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(54) Title: ADENOVIRUS PARTICLES WITH ENHANCED INFECTIVITY OF DENDRITIC CELLS AND PARTICLES WITH DECREASED INFECTIVITY OF HEPATOCYTES

(57) Abstract: Provided are adenovirus vectors and the production of such vectors. In particular, adenoviruses with modified or heterologous fiber proteins for targeting to dendritic cells are provided.





ADENOVIRUS PARTICLES WITH ENHANCED INFECTIVITY OF DENDRITIC CELLS AND PARTICLES WITH DECREASED INFECTIVITY OF HEPATOCYTES

Work described and claimed herein was supported by Department of Defense Prostate Cancer Research Program DAMD17-01-1-0098

Department of Defense Breast Cancer Research Program DAMD17-01-1-0391. The government has certain rights in such subject matter.

RELATED APPLICATIONS

Benefit of priority is claimed to U.S. provisional application Serial

No. 60/459,000, filed March 28, 2003, entitled "DETARGETING OF
ADENOVIRAL PARTICLES AND USES THEREOF", to Daniel J. Von
Seggern, and to U.S. provisional application Serial No. 60/467,500, filed
May 1, 2003, entitled "PSEUDOTYPED ADENOVIRAL VECTORS WITH
ENHANCED INFECTIVITY TOWARDS DENDRITIC CELLS", to Daniel J.

15 Von Seggern.

This application also is related to U.S. application Serial No. (attorney docket number 22908-1239), filed the same day herewith, entitled "PSEUDOTYPED ADENOVIRAL VECTORS WITH ENHANCED INFECTIVITY TOWARDS DENDRITIC CELLS," to Daniel J. Von Seggern.

- This application also is related to U.S. application Serial No. 10/403,337, filed March 27, 2003 and U.S. application Serial No. 10/351,890, filed January 24, 2003, to Michael Kaleko, Glen R. Nemerow, Theodore Smith and Susan C. Stevenson, entitled "FIBER SHAFT MODIFICATIONS FOR EFFICIENT TARGETING". This application also is related to U.S.
- 25 provisional application Serial No. 60/350,388, filed January 24, 2002, entitled "FIBER SHAFT MODIFICATIONS FOR EFFICIENT TARGETING," to Susan C. Stevenson, Michael Kaleko, Theodore Smith and Glen R. Nemerow, and to U.S. provisional application Serial No. 60/391,967, filed June 26, 2002, entitled "FIBER SHAFT MODIFICATIONS FOR
- 30 EFFICIENT TARGETING," to Stevenson, Susan C., Kaleko, Michael,

Smith, Theodore and Nemerow, Glen R. This application also is related to International PCT application No. PCT/USO3/02295, filed January 24, 2003, entitled "FIBER SHAFT MODIFICATIONS FOR EFFICIENT TARGETING," to Michael Kaleko, Glen R. Nemerow, Theodore Smith and Susan C. Stevenson.

Where permitted, the subject matter of each of these applications, provisional applications and international applications is incorporated by reference herein.

FIELD OF INVENTION

The present invention generally relates to the field of adenoviral vectors and the production of such vectors. Targeted and detargeted adenoviral vectors are provided. In particular, adenoviral vectors targeted to dendritic cells are provided.

BACKGROUND

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The immune system is designed to eradicate a large number of pathogens, as well as tumors, with minimal immunopathology. When the immune system becomes defective, however, numerous disease states result. Immunotherapy is an emerging treatment modality that seeks to harness the power of the human immune system to treat disease.

Immunotherapy is designed either to enhance the cellular immune response in subjects with diseases characterized by immunosuppression and/or to suppress the cellular immune response in subjects with diseases characterized by an overactive cellular immune response and/or to mount an immune response against pathogens or tumors. Improved immunotherapeutic protocols are needed.

In addition, despite the extensive characterization of numerous infectious agents and the availability of vaccines, new vaccines are needed to protect against or ameliorate diseases, such as tuberculosis, malaria (Plebanski *et al.* (2002) *J. Clin. Invest. 110*:295-301), and a large

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number of viruses including human immunodeficiency virus (HIV), herpes simples virus (HSV), human papilloma virus (HPV), Epstein-Barr virus (EBV), hepatitis C virus (HCV), respiratory syncytial virus (RSV), parainfluenza viruses and human metapneumovirus (Letvin (2002) *J. Clin. Invest. 110*:145-151; Murphy and Collins (2002) *J. Clin. Invest. 110*:21-27), caused by many clinically-relevant pathogens. Athough vaccines have been developed for influenza and anthrax, more effective vaccines to prevent or reduce the severity of the diseases caused by these agents are needed (see, *e.g.*, Palese and Garcia-Sastre (2002) *J. Clin. Invest. 110*:9-13; Leppla *et al.* (2002) *J. Clin. Invest. 110*:141-144; Steinman and Pope (2002) *J. Clin. Invest. 109*:1519-1526).

Vaccines and immunotherapy have been used to eliminate or a wide variety of cancerous cells and tumors to thereby effect treatment of cancer. Many human cancers are associated with the expression of specific proteins, such as tumor antigens, thus providing a means of identifying cancerous cells from normal cells, and providing a target for immunotherapy. The immune system is capable of recognizing these tumor antigens and eliciting an immune response directed against cells displaying the tumor antigen (van der Bruggen et al. (1991) Science 254:1643-1647; Sahin et al. (1995) Proc. Natl. Acad. Sci. U.S.A. 92:11810-11813; Kaplan et al. (1998) Proc. Natl. Acad. Sci. U.S.A. 95:7556-7561). The identification of these tumor antigens has led to the development of vaccine and immunotherapeutic approaches for the treatment of cancer (Scanlan and Jäger (2001) Breast Cancer Res. 3:95-98; Yu and Restifo (2002) J. Clin. Invest. 110:28-94).

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There, however, remain numerous challenges in the development of effective immunotherapies. These include, for example, a need for (i) enhancing antibody and T cell-mediated immune memory,

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(iii) enhancing T cell responses (CD4+ T helper cells and CD8+ CTLs), (iii) establishing mucosal immunity, which is important for vaccination against many sexually transmitted diseases, (iv) development of vaccines that can diminish the immune response, which is important for the treatment of autoimmune diseases (Steinman and Pope (2002) *J. Clin. Invest. 109*:1519-1526) and (v) others.

Most, if not all, adenoviral vector-mediated gene therapy strategies aim to transduce a specific tissue, such as a tumor or an organ, or a specific cell type, cells as immune cells. Systemic delivery will require ablation of the normal virus tropism as well as addition of new specificities. Multiple interactions between adenoviral particles and the host cell are required to promote efficient cell entry (Nemerow (2000) Virology 274:1-4). An adenovirus entry pathway is believed to involve two separate cell surface events. First, the adenoviral fiber knob mediates attachment of the adenovirus particle to a target cell through a high affinity interaction with a specific cell-surface receptor, which is the coxsackie-adenovirus receptor (CAR) for most, but not all, serotypes of adenovirus. A subsequent association of penton with cell surface integrins $\alpha_{\nu}\beta_{3}$ and $\alpha_{\nu}\beta_{5}$, which act as co-receptors, potentiates virus internalization. Although there are a plurality of adenoviral fiber receptors, in addition to CAR, that interact with subgroup B (e.g., Ad3) and subgroup C (e.g., Ad5) adenoviruses, both subgroups appear to require interaction with integrins for internalization.

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The role of CAR interaction for *in vivo* gene transfer is not clear.

25 CAR ablation does not change biodistribution and toxicity of adenoviral vectors *in vivo* (Alemany *et al.* (2001) *Gene Therapy 8*:1347-1353; U.S. patent application No. 09/870,203, filed May 30, 2001, and published as U.S. Published application No. 20020137213). Published studies have described conflicting results (Alemany *et al.* (2001) *Gene Therapy*

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8:1347-1353; Leissner et al. (2001) Gene Therapy 8:49-57; Einfeld et al. (2001) J. Virology 75:11284-11291). For example, it has been shown that vectors containing an S408E mutation in the Ad5 fiber AB loop yield efficient liver transduction in mice, despite having greatly reduced transduction efficiencies on cells in culture (see, Leissner et al. (2001) Gene Therapy 8:49-57). In contrast, vectors containing a more extensive fiber AB loop mutation showed a 10-fold reduction in liver gene expression (see, Einfeld et al. (2001) J. Virology 75:11284-11291).

A doubly ablated adenovirus has been prepared by modifying the CAR binding region in the fiber loop and the integrin binding region in the penton base (Einfeld *et al.* (2001) *J. Virology 75*:11284-11291). This doubly ablated adenovirus, lacking CAR and integrin interactions, was reported not only to lack *in vitro* transduction of various cell types but also to lack *in vivo* transduction of liver cells. Specifically, the doubly ablated adenovirus was reported to have a 700-fold reduction in liver transduction when compared to the non-ablated adenovirus. These results, however, were not reproduced by others.

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For many applications, the most clinically useful adenoviral vector would be deliverable systemically, such as into a peripheral vein, and would be targeted to a desired location in the body or a desired cell type, and would not have undesirable side effects resulting from targeting to other locations. *In vivo* adenoviral vector targeting is a major goal in gene therapy and a significant effort has been focused on developing strategies to achieve this goal. Successful targeting strategies would direct the entire vector dose to the appropriate site and would be likely to improve the safety profile of the vector by permitting the use of lower, less toxic vector doses, which potentially also can be less immunogenic. Thus, there is a need to develop adenoviruses that are fully detargeted *in vivo* for use as a base vector for producing redirected adenoviruses.

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Therefore, among the objects herein, it is an object to provide detargeted adenoviral vectors, methods for preparation thereof, and uses thereof. Furthermore, it is in an object herein to provide immunotherapeutic methods and compositions therefor.

5 SUMMARY

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Provided are immunotherapeutic methods and compositions that have immunotherapeutic activity. In particular, adenoviral vectors that deliver antigens to dendritic cells for processing and presentation to T cells are provided. Delivery of antigens to dendritic cells has preventive, diagnostic and therapeutic applications.

Detargeted and fully detargeted adenoviral particles from serotype C, such as adenovirus 2 and adenovirus 5, adenovirus vectors from which such particles are produced, methods for preparation of the vectors and particles and uses of the vectors and particles are provided. Retargeted particles also are provided.

The particles are detargarted from binding to certain native receptors (e.g., coxsackie-adenovirus receptor (CAR) for Ad5 and Ad2), and can be targeted to receptors expressed on dendritic cells. In addition, among the viral particles provides are particles that do not bind to or exhibit reduced binding to HSP (Heparin Sulfate Proteoglycans; also referred to as heparin sulfate glycosaminoglycans), and, hence, exhibit reduced or no binding to hepatocytes, which express HSPs.

Provided are the adenoviral particles and genomes encoding such particles and/or genomes (viral nucleic acid molecules), cell lines and methods for producing such particls. In particular genomes that encode Ad5 particles or other type C viral particles that express fibers from adenovirus subgroup D or subgroup B, such as Ad19p, Ad30, Ad37, Ad16 and Ad35 (or that express modified fibers thereof) are provided. The fibers are modified to permit incorporation into the particle. The viral

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particles provided herein exhibit reduced binding to hepatocytes and hence reduced liver toxicity.

Adenoviral particles that contain a heterologous fiber or a portion thereof, whereby binding of the viral particle to dendritic cells is increased and binding to heparin sulfate proteoglycans (HSP) and to CAR is reduced or eliminated compared to a particle that expresses its native fiber are provided. In these particles, the adenoviral (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and the fiber is from an adenovirus subgroup D, such as Ad19p. In another embodiment, the heterologous fiber is from Ad30. In other embodiments, the fiber is chimeric and comprises an N-terminal portion from a fiber of a subgroup C Ad virus; and the N-terminal portion is sufficient to increase incorporation into the particle compared to in its absence. For example, the fiber can be from an adenovirus Ad19p, Ad30, Ad37, Ad16 or Ad35 virus. The fiber protein can additionally include one or more further modifications that reduce or eliminate interaction of the resulting fiber with one or more cell surface proteins, such as CAR, in addition to HSP.

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The adenoviral particle also can include a mutation in the CAR-binding region of the capsid and/or a mutation in the $\alpha_{\rm v}$ integrin-binding region of the capsid, whereby binding to the integrin is eliminated or reduced. The CAR-binding region of the capsid that is modified can be on a fiber knob.

In some embodiments, the chimeric fiber contains at least a sufficient number of amino acids of Ad19p fiber set forth as SEQ ID No. 34 to target a particle to dendritic cells, and optionally to reduce or eliminate binding of the particle to HSP. For example, the Ad19p fiber is modified by replacing at least the N-terminal 15, 16 or 17 amino acids with the 15, 16 or 17 amino acids of an Ad2 or Ad5 fiber. In other embodiments, the chimeric fiber contains at least a sufficient number of

amino acids of Ad30 fiber set forth as SEQ ID No. 36 to target a particle to dendritic cells, and optionally to reduce or eliminate binding of the particle to HSP. For example, the Ad30 fiber is modified by replacing at least the N-terminal 15, 16 or 17 amino acids with the 15, 16 or 17 amino acids of an Ad2 or Ad5 fiber.

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Hence, also provided are methods for making and using the adenoviral particles that express the modified fibers and combinations of modified fibers and modified penton. With the fiber shaft modifications, particularly in combination with the fiber knob modifications and the penton modifications, the adenovirus particles are ablated for binding to their natural cellular receptor(s), *i.e.*, they are detargeted. In addition, by selection of a subgroup D fiber, the resulting particles are targeted to dendritic cells. The particles also can be "retargeted" to a specific cell type through the addition of a ligand to the virus capsid, which causes the virus to bind to and infect such cell. The ligand can be added, for example, through genetic modification of a capsid protein gene.

The nucleic acids, proteins, adenoviral particles and adenoviral vectors have a variety of uses. These include *in vivo* and *in vitro* uses to target nucleic acid to particular cells and tissues, for therapeutic purposes, including gene therapy, and also for the identification and study of cell surface receptors and identification of modes of interaction of viruses with cells.

Nucleic acids encoding the capsid proteins, including the fibers are also provided. The nucleic acids can be provided as vectors, particularly as adenovirus vectors. Many adenoviral vectors are known and can be modified as needed in accord with the description herein. Adenoviral vectors include, but are not limited to, early generation adenoviral vectors, such as E1-deleted vectors, gutless adenoviral vectors and replication-conditional adenoviral vectors, such as oncolytic adenoviral

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vectors. The adenovirus vectors also can include heterologous nucleic acids that encode or provide products, such as tumor antigens and antigens from pathogens that induce an immunotherapeutic response whereby infection with such pathogen is prevented or the symptoms of infection reduced. Heterologous nucleic acid can encode a polypeptide or comprise or encode a regulatory sequence, such as a promoter or an RNA, including RNAi, small RNAs, other double-stranded RNAs, antisense RNA, and ribozymes. Promoters include, for example, constitutive and regulated promoters and tissue specific promoters, including tumor specific promoters. The promoter can be operably linked, for example, to a gene of an adenovirus essential for replication.

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Cells containing the nucleic acid molecules and cells containing the vectors are also provided. Such cells include packaging cells. The cells can be prokaryotic or eukaryotic cells, including mammalian cells, such as primate cells, including human cells.

Also provided are adenoviral particles that contain the modified capsid proteins provided herein. The particles have increased tropism for dendritic cells, and also exhibit altered interaction or binding with HSP compared to particles that do not contain the modified capsid proteins. In addition to altered binding to HSP and dendritic cells, the particles can include further modifications, such as capsid proteins with altered interaction with other receptors as described above. In particular, the particles can have altered, typically reduced or eliminated, interaction with CAR, $\alpha_{\rm v}$ integrin and/or other receptors. The mutations include mutations in the fiber knob, penton and hexon. Exemplary fiber knob mutations are mutations in the AB loop or CD loop, such as KO1 or KO12. Such mutations include, for example, PD1, KO1, KO12 and S* (see, e.g., U.S. provisional application Serial No. 60/459,000, and copending U.S. application Serial No. 10/351,890). In addition, the particles can include

additional ligands for retargeting to selected receptors. The adenoviral particles can be from any serotype and subgroup.

Methods for expressing heterologous nucleic acids in a cell are provided. In these methods an adenoviral vector provided herein is transduced into a cell to deliver the nucleic acid and/or encoded products. Transduction can be effected *in vivo* or *in vitro* or *ex vivo*, and can be for a variety of purposes including study of gene expression and genetic therapy. The cells can be prokaryotic cells, but typically are eukaryotic cells, including mammalian cells, such as primate, including human cells.

The cells can be of a specific type, such as a tumor cell or a cell in a particular tissue.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a plasmid map for pSKO1.

Figure 2 is a plasmid map for pNDSQ3.1KO1.

Figures 3A-3C are plasmid maps of pAdmireRSVnBg (Fig. 3A), pSQ1 (Fig. 3B) and pSQ1KO12 (Fig. 3C).

Figure 4 is a plasmid map for pSQ1PD1.

Figures 5A-5B are plasmid maps of pSQ1FKO1PD1 (Fig. 5A) and pSQ1KO12PD1 (Fig. 5B).

Figure 6 shows *in vitro* transduction efficiency of A549 cells using adenoviral vectors containing fiber AB loop knob and/or penton, PD1 mutations. The following adenoviral vectors were used in these studies: Av1nBg, Av1nBgFKO1, referred to as FKO1, Av1nBgPD1, referred to as PD1, and Av1nBgFKO1PD1 that is referred to as FKO1PD1.

25 Figure 7A-7B shows *in vivo* adenoviral-mediated liver gene expression (Fig. 7A) and hexon DNA content (Fig. 7B) using adenoviral vectors containing fiber AB loop knob and/or penton, PD1 mutations. The following adenoviral vectors were used in these studies: Av1nBg, Av1nBgFKO1, referred to as FKO1, Av1nBgPD1, referred to as PD1,

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Av1nBgFKO1PD1, referred to as FKO1PD1, Av1nBgKO12, referred to as KO12, and Av1nBgKO12PD1 that is referred to as KO12PD1.

Figure 8 is a plasmid map for pFBshuttle(EcoRI).

Figure 9 is a plasmid map for pSQ1HSP.

5 Figure 10 is a plasmid map for pSQ1HSPKO1.

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Figure 11 is a plasmid map for pSQ1HSPPD1.

Figure 12 is a plasmid map for pSQ1HSPKO1PD1.

Figures 13A-13C show the transduction efficiency of A549 and HeLa cells using adenoviral vectors containing fiber shaft, knob and/or penton mutations. Fig. 13A shows the dose response for the transduction efficiency of A549 cells. Fig. 13B shows the transduction efficiency of HeLa cells at 2000 ppc. Figure 13C shows the competition analysis of adenoviral vectors containing fiber shaft mutations.

Figures 14A-14B shows the influence of fiber shaft mutations on *in vivo* adenoviral-mediated liver gene expression (Fig. 14A) and hexon DNA content (Fig. 14B).

Figures 15A-15B are plasmid maps of pSQ1HSPRGD (Fig. 15A) and pSQ1HSPKO1RGD (Fig. 15B).

Figure 16 shows that insertion of a RGD targeting ligand can restore transduction of the vectors containing the HSP binding shaft S* mutation.

Figures 17A-17B are plasmid maps of pSQ1AD35Fiber (Fig. 17A) and pSQ1Ad35FcRGD (Fig. 17B).

Figures 18A-18B are maps of plasmids encoding 35F chimeric fibers. Fig. 18A is a plasmid map of pSQ135T5H, and Fig. 18B is a plasmid map of pSQ15T35H.

Figure 19 shows the results of an *in vitro* analysis of Ad5 vectors containing Ad35 fibers and derivatives thereof.

Figure 20 shows the results of an *in vivo* analysis of Ad5 vectors containing Ad35 fibers and derivatives thereof.

Figures 21A-21B are plasmid maps of pSQ1Ad41sF (Fig. 21A) and pSQ1Ad41sFRGD (Fig. 21B).

Figure 22 shows the results of an *in vivo* analysis of Ad5 vectors containing Ad41 short fiber.

Figure 23 shows the *in vitro* analysis of Ad5 based vectors containing the Ad41 short fiber which has been re-engineered to contain a cRGD ligand in the HI loop.

Figure 24 shows enhanced transduction of AE1-2a cells with the Av3nBgFKO1 detargeted adenoviral vector using hexadimethrine bromide (HB), protamine sulfate (PS) and poly-lysine-RGD (K14) or the anti-penton-TNFα bifunctional protein (αpen-TNF).

Figure 25 shows ablation of HSP interaction decreases adenoviral-mediated gene transfer to other organs.

Figure 26 shows *in vivo* liver transduction with adenoviral vectors which encode for β -galactosidase and contain various mutations to the fiber and/or penton proteins. Results are plotted as percent transduction as compared to wild type. Two different methods for determining the level of transduction are shown for each vector.

Figure 27 shows the adenoviral vector biodistribution to the liver and tumor for the vectors containing the S*, KO1S*, and 41sF fibers.

DETAILED DESCRIPTION

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- A. DEFINITIONS
- B. Adenovirus-cell interactions
 - 1. Fiber protein
 - 2. Pseudotyping
- C. Dendritic cell targeting
 - 1. Dendritic cells
 - 2. Dendritic cell therapies
 - 3. Targeting adenoviral particles to dendritic cells
 - a. Fiber substitution
 - b. Efficient targeting

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- 4. Additional modifications
- D. Adenovirus vector detargating
- E. Nucleic acids, adenoviral vectors and cells containing the nucleic acids and cells containing the vectors
 - 1. Preparation of viral particles
 - 2. Adenoviral vectors and particles
 - a. Gutless vectors
 - b. Oncolytic vectors
 - 3. Packaging
- 4. Propagation and scale-up
 - F. Adenovirus expression vector systems
 - 1. Nucleic acid gene expression cassettes
 - 2. Promoters
 - G. Heterologous polynucleotides and therapeutic nucleic acids
- 15 H. Formulation and administration
 - 1. Formulation
 - 2. Administration
 - I. Diseases, disorders and therapeutic products
 - J. Examples

20 A. DEFINITIONS

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Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, Genbank sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it understood that such identifiers can change and particular information on the internet can come and go, but equivalent information is known and can be readily accessed, such as by searching the internet and/or appropriate databases. Reference thereto evidences the availability and public dissemination of such information.

As used herein, the term "adenovirus" or "adenoviral particle" is used to include any and all viruses that can be categorized as an adenovirus, including any adenovirus that infects a human or an animal, including all groups, subgroups, and serotypes. Depending upon the

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context reference to "adenovirus" can include adenoviral vectors. There are at least 51 serotypes of adenovirus that are classified into several subgroups. For example, subgroup A includes adenovirus serotypes 12, 18, and 31. Subgroup C includes adenovirus serotypes 1, 2, 5, and 6. Subgroup D includes adenovirus serotypes 8, 9, 10, 13, 15, 17, 19, 19p, 20, 22-30, 32, 33, 36-39, and 42-49. Subgroup E includes adenovirus serotype 4. Subgroup F includes adenovirus serotypes 40 and 41. These latter two serotypes have a long and a short fiber protein. Thus, as used herein an adenovirus or adenovirus particle is a packaged vector or genome.

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As used herein, "virus," "viral particle," "vector particle," "viral vector particle," and "virion" are used interchangeably to refer to infectious viral particles that are formed when, such as when a vector containing all or a part of a viral genome, is transduced into an appropriate cell or cell line for the generation of such particles. The resulting viral particles have a variety of uses, including, but not limited to, transferring nucleic acids into cells either *in vitro* or *in vivo*. For purposes herein, the viruses are adenoviruses, including recombinant adenoviruses formed when an adenovirus vector, such as any provided herein, is encapsulated in an adenovirus capsid. Thus, a viral particle is a packaged viral genome. An adenovirus viral particle is the minimal structural or functional unit of a virus. A virus can refer to a single particle, a stock of particles or a viral genome. The adenovirus (Ad) particle is relatively complex and may be resolved into various substructures.

Included among adenoviruses and adenoviral particles are any and all viruses that can be categorized as an adenovirus, including any adenovirus that infects a human or an animal, including all groups, subgroups, and serotypes. Thus, as used herein, "adenovirus" and

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"adenovirus particle" refer to the virus itself and derivatives thereof and cover all serotypes and subtypes and naturally occurring and recombinant forms, except where indicated otherwise. Included are adenoviruses that infect human cells. Adenoviruses can be wildtype or can be modified in various ways known in the art or as disclosed herein. Such modifications include, but are not limited to, modifications to the adenovirus genome that is packaged in the particle in order to make an infectious virus. Exemplary modifications include deletions known in the art, such as deletions in one or more of the E1a, E1b, E2a, E2b, E3, or E4 coding regions. Other exemplary modifications include deletions of all of the coding regions of the adenoviral genome. Such adenoviruses are known as "gutless" adenoviruses. The terms also include replication-conditional adenoviruses, which are viruses that preferentially replicate in certain types of cells or tissues but to a lesser degree or not at all in other types. For example, among the adenoviral particles provided herein, are adenoviral particles that replicate in abnormally proliferating tissue, such as solid tumors and other neoplasms. These include the viruses disclosed

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adenoviral particles that replicate in abnormally proliferating tissue, such as solid tumors and other neoplasms. These include the viruses disclosed in U.S. Patent No. 5,998,205 and U.S. Patent No. 5,801,029. Such viruses are sometimes referred to as "cytolytic" or "cytopathic" viruses (or vectors), and if they have such an effect on neoplastic cells, are referred to as "oncolytic" viruses (or vectors).

As used herein, the terms "vector," "polynucleotide vector," "polynucleotide vector construct," "nucleic acid vector construct," and "vector construct" are used interchangeably herein to mean any nucleic acid construct that can be used for gene transfer, as understood by those skilled in the art.

As used herein, the term "viral vector" is used according to its art-recognized meaning. It refers to a nucleic acid vector construct that includes at least one element of viral origin and can be packaged into a

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viral vector particle. The viral vector particles can be used for the purpose of transferring DNA, RNA or other nucleic acids into cells either in vitro or in vivo. Viral vectors include, but are not limited to, retroviral vectors, vaccinia vectors, lentiviral vectors, herpes virus vectors (e.g., 5 HSV), baculoviral vectors, cytomegalovirus (CMV) vectors, papillomavirus vectors, simian virus (SV40) vectors, Sindbis vectors, Semliki Forest virus vectors, phage vectors, adenoviral vectors, and adeno-associated viral (AAV) vectors. Suitable viral vectors are described, for example, in U.S. Patent Nos. 6,057,155, 5,543,328 and 5,756,086. The vectors provided herein are adenoviral vectors.

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As used herein, "adenovirus vector" and "adenoviral vector" are used interchangeably and are well understood in the art to mean a polynucleotide containing all or a portion of an adenovirus genome. An adenoviral vector, refers to nucleic encoding a complete genome or a modified genome or one that can be used to introduce heterologous nucleic acid when transferred into a cell, particularly when packaged as a particle. An adenoviral vector can be in any of several forms, including, but not limited to, naked DNA, DNA encapsulated in an adenovirus capsid, DNA packaged in another viral or viral-like form (such as herpes simplex, and AAV), DNA encapsulated in liposomes, DNA complexed with polylysine, complexed with synthetic polycationic molecules, conjugated with transferrin, complexed with compounds such as PEG to immunologically "mask" the molecule and/or increase half-life, or conjugated to a non-viral protein.

As used herein, oncolytic adenoviruses refer to adenoviruses that replicate selectively in tumor cells

As used herein, a variety of vectors with different requirements and purposes are described. For example, one vector is used to deliver particular nucleic acid molecules into a packaging cell line for stable

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integration into a chromosome. These types of vectors also are referred to as complementing plasmids. A further type of vector carries or delivers nucleic acid molecules in or into a cell line (e.g., a packaging cell line) for the purpose of propagating viral vectors; hence, these vectors also can be referred to herein as delivery plasmids. A third "type" of vector is the vector that is in the form of a virus particle encapsulating a viral nucleic acid and that is comprised of the capsid modified as provided herein. Such vectors also can contain heterologous nucleic acid molecules encoding particular polypeptides, such as therapeutic polypeptides or regulatory proteins or regulatory sequences to specific cells or cell types in a subject in need of treatment.

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As used herein, the term "motif" is used to refer to any set of amino acids forming part of a primary sequence of a protein, either contiguous or capable of being aligned to certain positions that are invariant or conserved, that is associated with a particular function. The motif can occur not only by virtue of the primary sequence, but also as a consequence of three-dimensional folding. For example, the adenovirus fiber is a trimer, hence the trimeric structure can contribute to formation of a motif. Alternatively, a motif can be considered as a domain of a protein, where domain is a region of a protein molecule delimited on the basis of function without knowledge of and relation to the molecular substructure, as, e.g., the part of a protein molecule that binds to a receptor. As shown herein, the motif KKTK constitutes a consensus sequence for fiber shaft interaction with HSP.

As used herein, cell therapy is a method of treatment involving the administration of live cells. Adoptive immunotherapy is a treatment process involving removal of cells from a subject, the processing of the cells in some manner *ex-vivo* and the infusion of the processed cells into the same or different subject as a therapy.

As used herein, a cell therapeutic refers to the compositions of cells that are formulated as a drug whose active ingredient is wholly or in part a living cell.

As used herein, immune cells are the subset of blood cells known as white blood cells, which include mononuclear cells such as lymphocytes, monocytes, macrophages and granulocytes.

As used herein, T-cells are lymphocytes that express the CD3 antigen.

As used herein, helper cells are CD4+ lymphocytes.

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As used herein, regulatory cells are a subset of T-cells, most commonly CD4+ T-cells, that are capable of enhancing or suppressing an immune response. Regulatory immune cells regulate an immune response primarily by virtue of their cytokine secretion profile. Some regulatory immune cells also can act to enhance or suppress an immune response by virtue of antigens expressed on their cell surface and mediate their effects through cell-to-cell contact. Th1 and Th2 cells are examples of regulatory cells.

As used herein, effector cells are immune cells that primarily act to eliminate tumors or pathogens through direct interaction, such as phagocytosis, perforin and/or granulozyme secretion, induction of apoptosis, etc. Effector cells generally require the support of regulatory cells to function and also act as the mediators of delayed type hypersensitivity reactions and cytotoxic functions. Examples of effector cells are B lymphocytes, macrophages, cytotoxic lymphocytes, LAK cells, NK cells and neutrophils.

As used herein, a professional antigen presenting cells (APC) include dendritic cells, B-cells and macrophages.

As used herein, the term "bind" or "binding" is used to refer to the binding between a ligand and its receptor, such as the binding of the Ad5

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knob domain with CAR (coxsackie-adenovirus receptor), with a K_d in the range of 10-2 to 10-15 mole/I, generally, 10^{-6} to 10^{-15} , 10^{-7} to 10^{-15} and typically 10^{-8} to 10^{-15} (and/or a K_a of 10^{5} - 10^{12} , 10^{7} - 10^{12} , 10^{8} - 10^{12} l/mole).

As used herein, specific binding or selective binding means that the binding of a particular ligand and one receptor interaction (k_a or K_{eq}) is at least 2-fold, generally, 5, 10, 50, 100 or more-fold, greater than for another receptor. A statement that a particular viral vector is targeted to a cell or tissue means that its affinity for such cell or tissue in a host or *in vitro* is at least about 2-fold, generally, 5, 10, 50, 100 or more-fold, greater than for other cells and tissues in the host or under the *in vitro* conditions.

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As used herein, the term "ablate" or "ablated" is used to refer to an adenovirus, adenoviral vector or adenoviral particle, in which the ability to bind to a particular cellular receptor is reduced or eliminated, generally substantially eliminated (*i.e.*, reduced more than 10-fold, 100-fold or more) when compared to a corresponding wild-type adenovirus. An ablated adenovirus, adenoviral vector or adenoviral particle also is said to be detargeted, i.e., the modified adenovirus, adenoviral vector or adenoviral particle does not possess the native tropism of the wild-type adenovirus. The reduction or elimination of the ability of the mutated adenovirus fiber protein to bind a cellular receptor as compared to the corresponding wild-type fiber protein can be measured or assessed by comparing the transduction efficiency (gene transfer and expression of a marker gene) of an adenovirus particle containing the mutated fiber protein compared to an adenovirus particle containing the wild-type fiber protein for cells having the cellular receptor.

As used herein, tropism with reference to an adenovirus refers to the selective infectivity or binding that is conferred on the particle by a capsid protein, such as the fiber protein and/or penton.

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As used herein, "penton" or "penton complex" is used herein to designate a complex of penton base and fiber. The term "penton" can also be used to indicate penton base, as well as penton complex. The meaning of the term "penton" alone should be clear from the context within which it is used.

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As used herein, the term "substantially eliminated" refers to a transduction efficiency less than about 11% of the efficiency of the wild-type fiber containing virus on HeLa cells. The transduction efficiency on Hela cells can be measured (see, e.g., Example 1 of U.S. Patent Application Serial No. 09/870,203 filed on May 30, 2001, and published as U.S. Published application No. 20020137213, and of International Patent Application No. PCT/EP01/06286 filed June 1, 2001, and published as WO 01/92299). Briefly, HeLa cells are infected with the adenoviral vectors containing mutated fiber proteins to evaluate the effects of fiber amino acid mutations on CAR interaction and subsequent gene expression. Monolayers of HeLa cells in 12 well dishes are infected with, for example, 1000 particles per cell for 2 hours at 37° C in a total volume of, for example, 0.35 ml of the DMEM containing 2% FBS. The infection medium is then aspirated from the monolayers and I ml of complete DMEM containing 10% FBS was added per well. The cells are incubated for an sufficient time, generally about 24 hours, to allow for β galactosidase expression, which is measured by a chemiluminescence reporter assay and by histochemical staining with a chromogenic substrate. The relative levels of β -galactosidase activity are determined using as suitable system, such as the Galacto-Light chemiluminescence reporter assay system (Tropix, Bedford, Mass.). Cell monolayers are washed with PBS and processed according to the manufacturer's protocol. The cell homogenate is transferred to a microfuge tube and centrifuged to remove cellular debris. Total protein concentration is

determined, such as by using the bicinchoninic acid(BCA) protein assay (Pierce, Inc., Rockford, III.) with bovine serum albumin as the assay standard. An aliquot of each sample is then incubated with the Tropix β -galactosidase substrate for 45 minutes in a 96 well plate. A

- luminometer is used determine the relative light units (RLU) emitted per sample and then normalized for the amount of total protein in each sample (RLU/ug total protein). For the histochemical staining procedure, the cell monolayers are fixed with 0.5% glutaraldehyde in PBS, and then were incubated with a mixture of 1 mg of
- 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) per ml, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide and 2 mM MgCl₂ in 0.5 ml of PBS. The monolayers are washed with PBS and the blue cells are visualized by light microscopy, such as with a Zeiss IDO3 microscope. Generally, the efficiency is less than about 9%, and typically is less than about 8%.

As used herein, the phrase "reduce" or "reduction" refers to a change in the efficiency of transduction by the adenovirus containing the mutated or heterologous fiber as compared to the adenovirus containing the wild-type fiber to a level of about 75% or less of the wild-type on HeLa cells. Generally, the change in efficiency is to a level of about 65% or less than wild-type. Typically it is about 55% or less. This system is able to rapidly analyze modified fiber proteins and/or modified penton proteins for desired tropism in the context of the viral particle.

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As used herein, the term "mutate" or "mutation" or similar terms refers to the deletion, insertion or change of at least one amino acid in the protein of interest (e.g. the part of the fiber shaft region interacting with HSP). The amino acid can be changed by substitution or by modification in a way that derivatizes the amino acid.

As used herein, the term "polynucleotide" means a nucleic acid molecule, such as DNA or RNA, that encodes a polynucleotide. The molecule can include regulatory sequences, and is generally DNA. Such polynucleotides are prepared or obtained by techniques known by those skilled in the art in combination with the teachings contained therein.

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As used herein, the term "viral vector" is used according to its artrecognized meaning. It refers to a nucleic acid vector construct that includes at least one element of viral origin and can be packaged into a viral vector particle. The viral vector particles can be used, for example, for transferring DNA into cells either *in vitro* or *in vivo*.

As used herein, adenoviral genome is intended to include any adenoviral vector or any nucleic acid sequence comprising a modified fiber protein. All adenovirus serotypes are contemplated for use in the vectors and methods herein.

As used herein, a packaging cell line is a cell line that is able to package adenoviral genomes or modified genomes to produce viral particles. It can provide a missing gene product or its equivalent. Thus, packaging cells can provide complementing functions for the genes deleted in an adenoviral genome (e.g., the nucleic acids encoding modified fiber proteins) and are able to package the adenoviral genomes into the adenovirus particle. The production of such particles require that the genome be replicated and that those proteins necessary for assembling an infectious virus are produced. The particles also can require certain proteins necessary for the maturation of the viral particle. Such proteins can be provided by the vector or by the packaging cell.

As used herein, detargeted adenoviral particles have ablated (reduced or eliminated) interaction with receptors with which native particles. It is understood that *in vivo* no particles are fully ablated such that they do not interact with any cells. Detargeted particles have

reduced, typically substantially reduced, or eliminated interaction with native receptors. For purposes herein, detargeted particles have reduced (2-fold, 5-fold, 10-fold, 100-fold or more) binding or virtually no binding to CAR or another native receptor. The particles still bind to cells, but the types of cells and interactions are reduced.

As used herein, pseudotyping describes the production of adenoviral vectors having modified capsid protein or capsid proteins from a different serotype from the serotype of the vector itself. One example, is the production of an adenovirus 5 vector particle containing an Ad37 or Ad35 fiber protein. This can be accomplished by producing the adenoviral vector in packaging cell lines expressing different fiber proteins.

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As used herein, receptor refers to a biologically active molecule that specifically or selectively binds to (or with) other molecules. The term "receptor protein" can be used to more specifically indicate the proteinaceous nature of a specific receptor.

As used herein, the term "heterologous polynucleotide" means a polynucleotide derived from a biological source other than an adenovirus or from an adenovirus of a different strain or can be a polynucleotide that is in a different locus from wild-type virus. The heterologous polynucleotide can encode a polypeptide, such as a toxin or a therapeutic protein. The heterologous polynucleotide can contain regulatory regions, such as a promoter regions, such as a promoter active in specific cells or tissue, for example, tumor tissue as found in oncolytic adenoviruses.

25 Alternatively, the heterologous polynucleotide can encode a polypeptide and further contain a promoter region operably linked to the coding region.

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As used herein, the term "cyclic RGD" (or cRGD) refers to any amino acid that binds to α_v integrins on the surface of cells and contains the sequence RGD (Arg-Gly-Asp).

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As used herein, the KO mutations refer to mutations in fiber that knock out binding to CAR. For example, a KO1 mutation refers to a mutation in the Ad5 fiber and corresponding mutations in other fiber proteins. In Ad5, this mutation results in a substitution of fiber amino acids 408 and 409, changing them from serine and proline to glutamic acid and alanine, respectively. As used herein, a KO12 mutation refers to a mutation in the Ad5 fiber and corresponding mutations in other fiber proteins. In Ad5, this mutation is a four amino acid substitution as follows: R512S, A515G, E516G, and K517G. Other KO mutations can be identified empirically or are known to those of skill in the art.

As used herein, PD mutations refer to mutations in the penton gene that ablate binding by the encoded to $\alpha_{\rm v}$ integrin by replacing the RGD tripeptide. The PD1 mutation exemplified herein results in a substitution of amino acids 337 through 344 of the Ad5 penton protein, HAIRGDTF (SEQ ID NO. 49), with amino acids SRGYPYDVPDYAGTS (SEQ ID NO. 50), thereby replacing the RGD tripeptide.

As used herein, reference to an amino acid in an adenovirus protein or to a nucleotide in an adenovirus genome is with reference to Ad5, unless specified otherwise. Corresponding amino acids and nucleotides in other adenovirus strains and modified strains and in vectors can be identified by those of skill in the art. Thus, recitation of a mutation is intended to encompass all adenovirus strains that possess a corresponding locus.

As used herein, tumor antigen refers to a cell surface protein expressed or located on the surface of tumor cells.

As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease are ameliorated or otherwise beneficially altered.

As used herein, a therapeutically effective product is a product that is encoded by heterologous DNA that, upon introduction of the DNA into a host, a product is expressed that effectively ameliorates or eliminates the symptoms, manifestations of an inherited or acquired disease or that cures said disease.

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As used herein, a subject is an animal, such as a mammal, typically a human, including patients.

As used herein, genetic therapy involves the transfer of heterologous DNA to the certain cells, target cells, of a mammal, particularly a human, with a disorder or conditions for which such therapy is sought. The DNA is introduced into the selected target cells in a manner such that the heterologous DNA is expressed and a therapeutic product encoded thereby is produced. Alternatively, the heterologous DNA may in some manner mediate expression of DNA that encodes the therapeutic product, it may encode a product, such as a peptide or RNA that in some manner mediates, directly or indirectly, expression of a therapeutic product. Genetic therapy may also be used to deliver nucleic acid encoding a gene product to replace a defective gene or supplement a gene product produced by the mammal or the cell in which it is introduced. The introduced nucleic acid may encode a therapeutic compound, such as a growth factor inhibitor thereof, or a tumor necrosis factor or inhibitor thereof, such as a receptor therefor, that is not normally produced in the mammalian host or that is not produced in therapeutically effective amounts or at a therapeutically useful time. The heterologous DNA encoding the therapeutic product may be modified prior to

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introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof.

As used herein, a therapeutic nucleic acid is a nucleic acid that encodes a therapeutic product. The product can be nucleic acid, such as a regulatory sequence or gene, or can encode a protein that has a therapeutic activity or effect. For example, therapeutic nucleic acid can be a ribozyme, antisense, double-stranded RNA, a nucleic acid encoding a protein and others.

As used herein, "homologous" means about greater than 25% nucleic acid sequence identity, such as 25%, 40%, 60%, 70%, 80%, 10 90% or 95%. If necessary the percentage homology will be specified. The terms "homology" and "identity" are often used interchangeably. In general, sequences are aligned so that the highest order match is obtained (see, e.g.: Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and 15 Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, 20 New York, 1991; Carillo et al. (1988) SIAM J Applied Math 48:1073). By sequence identity, the number of conserved amino acids are determined by standard alignment algorithms programs, and are used with default gap penalties established by each supplier. Substantially homologous nucleic acid molecules would hybridize typically at moderate 25 stringency or at high stringency all along the length of the nucleic acid or along at least about 70%, 80% or 90% of the full-length nucleic acid molecule of interest. Also contemplated are nucleic acid molecules that

contain degenerate codons in place of codons in the hybridizing nucleic acid molecule.

Whether any two nucleic acid molecules have nucleotide sequences that are at least, for example, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" can be determined using known computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson et al. (1988) Proc. Natl. Acad. Sci. USA 85:2444 (other programs include the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(I):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., et al., J Molec Biol 215:403 (1990); Guide to Huge Computers, 10 Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo et al. (1988) SIAM J Applied Math 48:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs include, DNAStar "MegAlign" program (Madison, WI) and the 15 University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI)). Percent homology or identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program (e.g., Needleman et al. 20 (1970) J. Mol. Biol. 48:443, as revised by Smith and Waterman ((1981) Adv. Appl. Math. 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for 25 non-identities) and the weighted comparison matrix of Gribskov et al. (1986) Nucl. Acids Res. 14:6745, as described by Schwartz and Dayhoff, eds., ATLAS OF PROTEIN SEQUENCE AND STRUCTURE, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of

3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide.

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As used herein, the term "at least 90% identical to" refers to percent identities from 90 to 99.99 relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polynucleotide length of 100 amino acids are compared, no more than 10% (i.e., 10 out of 100) of amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons can be made between a test and reference polynucleotides. Such differences can be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions. At the level of homologies or identities above about 85-90%, the result should be independent of the program and gap parameters set; such high levels of identity can be assessed readily, often without relying on software.

As used herein: stringency of hybridization in determining percentage mismatch is as follows:

- 1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C
- 2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C
- 3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C

Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, e.g., Sambrook, E.F. Fritsch, T. Maniatis, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), vol. 3, p. B.13, see,

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also, numerous catalogs that describe commonly used laboratory solutions). SSPE is pH 7.4 phosphate- buffered 0.18 M NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by T_m , which is a function of the sodium ion concentration and temperature ($T_m = 81.5^{\circ}$ C-16.6($log_{10}[Na^+]$) + 0.41(%G+C)-600/l)), so that the only parameters in the wash conditions critical to hybrid stability are sodium ion concentration in the SSPE (or SSC) and temperature.

It is understood that equivalent stringencies can be achieved using alternative buffers, salts and temperatures. By way of example and not limitation, procedures using conditions of low stringency are as follows (see also Shilo and Weinberg, *Proc. Natl. Acad. Sci. USA 78*:6789-6792 (1981)): Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μ g/ml denatured salmon sperm DNA (10X SSC is 1.5 M sodium chloride, and 0.15 M sodium citrate, adjusted to a pH of 7).

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Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10⁶ cpm ³²P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 40°C, and then washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which can be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

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By way of example and not way of limitation, procedures using conditions of moderate stringency include, for example, but are not limited to, procedures using such conditions of moderate stringency are as follows: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhart's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution and 5-20 X 10⁶ cpm ³²P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 55°C, and then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency which can be used are well-known in the art. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS.

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By way of example and not way of limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μ g/ml denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization mixture containing 100 μ g/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes before autoradiography. Other conditions of high stringency which can be used are well known in the art.

The term substantially identical or substantially homologous or similar varies with the context as understood by those skilled in the relevant art and generally means at least 60% or 70%, preferably means

at least 80%, 85% or more preferably at least 90%, and most preferably at least 95% identity.

As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

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As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound can, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

The methods and preparation of products provided herein, unless
otherwise indicated, employ conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, immunology, cell biology, cell culture and transgenic biology, which are within the skill of the art (see, e.g., Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY); Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel et al. (1992) Current Protocols in Molecular Biology, Wiley and Sons, New York; Glover (1985) DNA Cloning I and II, Oxford Press; Anand (1992) Techniques for the Analysis of Complex Genomes

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(Academic Press); Guthrie and Fink (1991) Guide to Yeast Genetics and Molecular Biology, Academic Press; Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Jakoby and Pastan, eds. (1979) Cell Culture. Methods in Enzymology 5 58, Academic Press, Inc., Harcourt Brace Jaovanovich, NY; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal (1984), A Practical Guide To Molecular Cloning; Gene Transfer Vectors For Mammalian Cells (J. H. 10 Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.); Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Hogan et al. (1986) Manipulating the Mouse Embryo, Cold Spring Harbor

В. Adenovirus-cell interactions

Laboratory Press, Cold Spring Harbor, N.Y.

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The ability of different subgroups of adenovirus to interact with, or not interact with, specific cell types and/or particular receptors can be exploited to produce adenoviruses with desired specificity. For example, adenovirus can be modified such that they are able to more efficiently target specific cell types and/or tissues. Adenovirus serotypes also can be modified to reduce or eliminate their interactions with a natural receptor and thereby reduce or eliminate the interaction of adenovirus with a particular cell type and/or tissue. Thus, provided herein are modifications of the viral capsid that alter the interaction of an adenovirus with its natural receptors and/or cell types and modifications that target an adenovirus to interact with other receptors and/or cell types. In particular, modifications that result targeting to dendritic cells are

provided. Also provided are modifications that result in reduction or ablation of the interaction of an adenovirus, particularly *in vivo*, with other cell types.

Different adenovirus serotypes infect different cell types, largely because their fibers bind distinct receptors (Defer et al. (1990) J. Virol. 5 64:3661-3673; Stevenson et al. (1995) J. Virol. 69:2850-2857; Arnberg et al. (2000) J. Virol. 74:42-48). For subgroup C viruses (including Ad2 and Ad5), coxsackievirus and adenovirus receptor (CAR) serves as the cellular receptor (Tomko and Philipson (1997) Proc. Natl. Acad. Sci. U.S.A. 94:3352-3356; Bergelson et al. (1997) Science 275:1320-1323). 10 While adenoviruses from several other subgroups also bind CAR (Roelvink et al. (1998) J. Virol. 72:7909-7915), infection and competition studies indicate that they use other proteins as primary receptors (Arnberg et al. (2000) J. Virol. 74:42-48; Huang et al. (1999) J. Virol. 73:2798-2802; Segerman et al. (2000) J. Virol. 74:1457-1467; Wu et al. (2001) Virology 15 279:78-89; Shayakhmetov et al. (2000) J. Virol. 74:2567-2584).

1. Fiber Protein

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The adenovirus fiber protein is a homotrimeric protein containing three polypeptides of 62 kDa. Ad fiber proteins are located at each of the twelve icosahedral vertices of the viral particle (Chroboczek *et al.* (1995) *Curr. Top. Microbiol. Immunol. 199*:163-200). The sequences of the fiber gene from a variety of serotypes including adenovirus serotypes 2 (Ad2), Ad5, Ad3, Ad12, Ad35, Ad40, and Ad41 are known. There are at least 21 different fiber genes in Genbank. Sequence analysis of fiber proteins from several different adenovirus serotypes (Hong *et al.* (1988) *Virology 167*:545-553; Kidd *et al.* (1990) *Virology 179*:139-150; Signäs *et al.* (1985) *J. Virol. 53*:672-678) and the crystal structure of Ad2 fiber (van Raaij *et al.* (1999) *Nature 401*:935-938) have identified three structural domains in the fiber. The N-terminal region of the fiber protein

interacts with the penton base proteins to anchor the fiber to the viral particle. The C-terminal knob region is responsible for mediating virus binding to host cells. These two regions are connected via a long, thin central shaft region, which contains a variable number of shaft repeats, each repeat being made up of 15 residues designated as a-o. The repeating domains of the fiber shaft are characterized by an invariant glycine or proline at position j and a conserved pattern of hydrophobic residues (van Raaij et al. (1999) Nature 401:935-938). A conserved stretch of amino acids which includes the sequence TLWT (SEQ ID No.

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10 46) marks the boundary between the repeating units of beta structure in the shaft and the globular head domain. The number of shaft repeats in Ad fiber depends on the adenoviral serotype. For example, Ad2 and Ad5 fiber proteins include 22 shaft repeats, while Ad3 contains only 5 repeats (Chroboczek *et al.* (1995) *Curr. Top. Microbiol. Immunol. 199*:163-200).

The C-terminal fiber knob mediates attachment to CAR, which is a 46 kDa protein of the immunoglobulin superfamily that is found on many different cell types (Bergelson *et al.* (1997) *Science 275*:1320-1323). A crystal structure of the Ad12 fiber in complex with CAR demonstrates that sequences in the fiber knob, specifically the AB loop, interact with the first lg-like domain of CAR (Bewley *et al.* (1999) *Science 286*:1579-1583). Following attachment to CAR, binding of the Ad penton base protein to $\alpha_{\rm v}$ integrins enables internalization and penetration of the virus into the cell.

Adenovirus interactions with specific cell types are also influenced by the capacity to bind HSP. As noted, adenoviruses having fiber shafts that do not interact with HSP include (a) adenoviruses of subgroup B, e.g., Ad3, Ad35, Ad7, Ad11, Ad16, Ad21, (b) adenoviruses of subgroup F, e.g., Ad40 and Ad41, specifically the short fiber, and (c) adenoviruses of subgroup D, which includes adenovirus serotype 8, 9, 10, 13, 15, 17,

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19, 20, 22-30, 32, 33, 36-39, and 42-49. Serotype 19 has variants. Ad19p is a nonpathogenic variant of Ad19 (Arnberg *et al.* (1998) *Virology* 227:239-244) while Ad19a, along with Ad8 and Ad37, are major causes of EKC. Ad19a and Ad37 have identical fiber proteins (Arnberg *et al.* (1998) *Virology* 227:239-244) and have similar tropism *in vivo*.

2. Pseudotyping

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Adenoviral vectors can be modified for targeting specific tissue and/or cell types through a variety of modifications, including modifications to the viral capsid, particularly to the fiber protein.

10 Modifications provided herein include, but are not limited to, pseudotyping of the viral particle with heterologous and/or chimeric fiber protein.

Fibers that use non-CAR receptors can direct infection of a variety of different cell types (Shayakhmetov *et al.* (2000) *J. Virol.* 74:2567-2584; Von Seggern *et al.* (2000) *J. Virol.* 74:354-362; Law and Davidson (2002) *J. Virol.* 76:656-661; Havenga *et al.* (2002) *J. Virol.* 76:4612-4620; Gall *et al.* (1996) *J. Virol.* 70:2116-2123; Chillon *et al.* (1999) *J. Virol.* 73:2537-2540), thus providing a means for adenovirus vector targeting. Adenovirus packaging cell systems allow generation of viral particles with essentially any desired fiber protein by transcomplementation of a fiber-deleted virus (Von Seggern *et al.* (2000) *J. Virol.* 74:354-362; Von Seggern *et al.* (1999) *J. Virol.* 73:1601-1608). This technology, referred to as psuedotyping, allows generation of targeted viral particles that can be used to study tropism *in vitro* and *in*

As described herein, psuedotyping can be used to identify fibers from subgroup D adenoviruses that confer enhanced infectivity of dendritic cells. Fiberless adenovirus vectors can be pseudotyped with fiber proteins from different serotypes to generate adenovirus particles

fibers do not bind to the producer cells normally used for Ad growth.

vivo, as well as permitting construction and propagation of viruses whose

with heterologous fiber proteins. Pseudotyping can be accomplished, for example, by expression in cells that contain expression plasmids encoding the fibers for pseudotyping. These vectors and plasmids can be generated as described herein or by any method known to those of skill in the art.

Accordingly, provided herein are modified fibers for targeting and detargeting and methods of making such fiber proteins and adenoviruses containing the fiber proteins. Among the cell types provided herein for adenovirus targeting are dendritic cells.

10 C. Dendritic cell targeting

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Denditric cells have numerous physiological features that render them desirable targets for immunotherapeutic approaches. Dendritic cells pick up antigens and migrate from the tissues of the body to the lymphoid tissues. There these cells present the antigens in lymphoid organs by displaying a foreign epitope bound to an MHC protein and trigger humoral and cellular immune responses. Dentritic cells have the ability to distinguish different types of pathogens, such as viruses, bacteria, fungi, and switch on specifically targeted immune-response genes against them. They are antigen-presenting cells that stimulate T lymphocytes into attacking infection. Hence delivery of heterologous antigens for presentation by dendritic cells provides a means for triggering humoral and cellular immune responses against such antigens. Also as noted, expression of particular products in dendritic cells also can function to inhibit or decrease in inappropriate or undesirable immune response, such occurs in allergies, autoimmune diseases and inflammatory responses.

1. Dendritic Cells

Dendritic cells (abbreviated DCs), which have a variety of important physiological features in the immune system, can serve as targets for immunotherapy and vaccine development. Dendritic cells play an

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important role in establishing an immune response. Dendritic cells are found in T-cell rich areas of the lymphoid tissues where they present antigen to T cells to stimulate the adaptive immune response (Janeway and Travers (1997) Immunobiology: the immune system in health and disease, third edition, Current Biology Ltd., New York, N.Y.).

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As an antigen presenting cell, the role of the dendritic cell is to capture foreign and self antigens, process them into peptides, and present the peptides in the context of MHC (major histocompatibility complex) proteins to T lymphocytes. Dendritic cells are highly specialized and efficient APCs and they control the magnitude, quantity, and memory of the adaptive immune responses that they trigger (Steinman and Pope (2002) *J. Clin. Invest.* 109:1519-1526). The T cells activated by dendritic cells presenting antigen include, T-helper CD4 + cells, particularly cells designated Th1, and CD8 + cytotoxic T lymphocytes (CTLs). Activated Th1 cells produce IFN-y and induce proliferation and antibody production of antigen-specific B lymphocytes. CTLs activated by dendritic cells kill cells displaying antigen (such as virus-infected cells) by releasing cytotoxic granules into the cell (see, e.g., Steinman and Pope (2002) *J. Clin. Invest.* 109:1519-1526).

As noted, dendritic cells express high levels of MHC molecules for antigen presentation rendering them highly efficient APCs. In addition they also express a high level of co-stimulatory molecules, which are important for enhancing an immune response. Dendritic cells also produce a wide array of immunostimulatory cytokines (Scanlan and Jäger (2001) *Breast Cancer Res. 3*:95-98) and there potentiate and participate in immune responses, and have been used as targets for vaccine development. Engineering of dentritic cells to express a tumor antigen has been pursued as an approach to tumor immunotherapy. For example, genetically modified dendritic cells that express particular antigens, such

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as tumor antigens, can be used as vaccines. In addition, genetic therapy that targets such cells *in vivo* can be used to generate APCs *in vivo* in immunotherapeutic methods.

Dendritic cells also are capable of diminishing an immune response.

Dendritic cells can be exploited to aid in vaccination against autoimmunity, allergy and transplantation rejection, all of which result from an uncontrolled or unchecked immune response (Hawiger *et al.* (2001) *J. Exp. Med. 194*:769-779; Steinman *et al.* (2003) *Annual Rev. Immunol. 21*:685-711). For example, dendritic cells appear to be

important for peripheral T cell tolerance (see, e.g., Steinman et al. (2000) J. Exp. Med. 191:411-416). Tolerance, or unresponsiveness to an antigen, is critical for avoidance of autoimmunity. Dendritic cells are capable of inducing significant antigen-specific tolerance in peripheral lymphoid tissues (Hawiger et al. (2001) J. Exp. Med. 194:769-779), and also are capable of inducing tolerance to transplantation antigens (see, Fu

et al. (1996) Transplantation 62:659-665) and contact allergens (se, Steinbrink et al. (1997) J. Immunol. 159:4772-4780).

Thus, vaccine and immmunotherapeutic strategies involving dendritic cells are important for the treatment of a variety of clinically important autoimmune and related diseases, including systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis, insulin-dependent diabetes mellitus and Graves' disease, as well as for vaccination or treatment of cancers and diseases caused by pathogens.

2. Dendritic Cell Therapies

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Different methods for delivery of the antigen gene to dendritic cells have been explored, but these generally require *ex vivo* manipulation of cells, including transfection, and then infusion of the cells. This is complicated, expensive, and requires generation of patient-specific reagents.

Adenovirus can be used for dendritic cell therapies. The ability of adenovirus serotypes to infect specific cell types, such as dendritic cells, can in part be attributed to their interaction, or a lack of interaction, with CAR. For example, the requirement for high doses of Ad5 in dendritic cell (DC) transduction can be explained by the lack of CAR expression on dendritic cells (Linette et al. (2000) J. Immunol. 164:3402-3412; Tillman et al. (1999) J. Immunol. 162:6378-6383). Several approaches have been used to improve DC infection by adenoviruses. A bispecific antibody (Ab) which bound to the fiber knob as well as to CD40 (which is expressed on the surface of DCs) was used to target dendritic cells (Tillman et al. (1999) J. Immunol. 162:6378-6383). This study showed that the cells expressed sufficient a_{v} integrins for efficient infection by the DC-binding adenovirus. This approach requires that production of the viral vector and the antibody, and purifcation of the complex, in clinically acceptable forms. This presents problems with scale-up and manufacturing, and negates many of the advantages of adenovirus vectors, most notably simple production and purification, as well as vector stability.

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Therefore to overcome these limitations, provided herein are adenoviral vectors that have been modified for efficiently targeting dendritic cells. The adenoviral vectors can be used for targeting such cells *in vivo* and *ex vivo* for immunotherapy and *in vitro* for studying dendritic cell function.

3. Targeting Adenoviral Particles to Dendritic Cells

Numerous studies have shown that adenovirus (Ad)-mediated delivery to dendritic cells can lead to anti-tumor response, but the Ad vectors generally used in gene therapy are based on a serotype (Ad5) that infects dendritic cells very inefficiently. After *in vivo* Ad administration, infection of a fairly small number of dendritic cells has been directly

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demonstrated (Zhang et al. (2001) Mol. Therapy 3:697-707; Oberholzer et al. (2002) J. Immunol. 168:3412-3418; Jooss et al. (1998) J. Virol. 72:4212-4223) and appears to be largely responsible for the cellular immune response observed (Zhang et al. (2001) Mol. Therapy 3:697-707; Jooss et al. (1998) J. Virol. 72:4212-4223). Since Ad5 infects dendritic cells poorly (Dietz et al. (1998) Blood 91:393-398; Wan et al. (1997) Human Gene Ther. 8:1355-1363; Jonuleit et al. (2000) Gene Therapy 7:249-254; Linette et al. (2000) J. Immunol. 164:3402-3412; Tillman et al. (1999) J. Immunol. 162:6378-6383) high multiplicities of infection are required.

Most testing has been done using primary cultures of dendritic cells derived from peripheral blood or bone marrow by incubation with cytokines, usually GM-CSF and IL-4 (Inaba *et al.* (1998) Isolation of dendritic cells *In* Current Protocols in Immunology, John Wiley & Sons, Inc. Philadelphia, 3.7.1-3.7.15). *Ex vivo* infection of dendritic cells, followed by re-infusion, has been found to generate effective anti-tumor responses (Wan *et al.* (1997) *Human Gene Ther.* 8:1355-1363; Linette *et al.* (2000) *J. Immunol.* 164:3402-3412; Inoue *et al.* (1999) *Immunol.* Lett. 70:77-81; Sonderbye *et al.* (1998) *Exp. Clin. Immunogenet.* 15:100-20 111; Ranieri *et al.* (1999) *J. Virol.* 73:10416-10425; Ribas *et al.* (1997) Cancer Res. 57:2865-2869; Miller *et al.* (2000) *Human Gene Ther.* 11:53-65). *Ex vivo* infection is not the ideal means for vaccination and immunotherapy.

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In addition, recombinant adenoviruses with fiber proteins from the subgroup B viruses Ad16 and Ad35 have been found to have an increased ability to infect human dendritic cells (Havenga *et al.* (2002) *J. Virol.* 76:4612-4620; Rea *et al.* (2001) *J. Immunol.* 166:5236-5244). Subgroup B viruses, however, appear to have a broad tropism. For example, they transduce a wide variety of cultured cell lines as well as

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primary cells from a number of different tissue types (Havenga *et al.* (2002) *J. Virol.* 76:4612-4620), evidencing such broad tropism. This apparent lack of cell-specificity (broad tropism) demonstrated by subgroup B indicates that pseudotyping Ad5 or Ad2 virsues with Ad subgroup B fibers is not advantageous.

a. Fiber Substitution

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It is shown herein that, contrary to reports in the literature, subgroup D viruses can target dendritic cells. Subgroup D viruses exhibit a narrower tropism than subgroup B viruses. It is shown herein that fibers from certain non CAR-using Ad serotypes, particularly Ad subgroup D viruses, effectively target receptors on dendritic cells.

Modified adenvirus particles can be generated by substituting dendritic cell-tropic fibers, such as the Subgroup D fibers, or portions thereof in place of the Ad subgroup C (or other Ad subgroup, including B and D, with a heterologous fiber), such as Ad5 or Ad2 fiber to produce degarteted (reduced binding to CAR, HSP) and retargeted (to dendritic cells) viral particles.

Any portion of the fiber can replaced with a portion of a subgroup D fiber, so long as the portion of the subgroup D fiber confers targeting to dendritic cells and the fiber assembles into the viral capsid. In one embodiment, the entire fiber protein is replaced with a subgroup D fiber. In another embodiment, the entire fiber, except for the N-terminus is replaced. For example, at least about 16 or 17 amino acids or more, up to about 60, 70, 80, 90, 100 or more amino acids of N-terminus of the native fiber is retained to aid in the incorporation of the fiber into the native particle.

Included in the modified adenoviruses provided herein are those with fiber protein from subgroup B and D, including, but not limited to Ad19p, Ad37, Ad30, Ad8, Ad9, Ad10, Ad13, Ad15, Ad17, Ad19, Ad20,

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Ad16, Ad35 and adenovirus serotypes 22-30, 32, 33, 36-39, and 42-49, expressed on adenoviral particles, particularly subgroup C particles. Among the modified capsid proteins are provided herein are those which include fibers containing the sequence of amino acids set forth in any of SEQ ID NOs. 32, 34, 36, 38 or 40; or a sequence of amino acids having 60%, 70%, 80%, 90%, 95% or greater sequence identity with a sequence of amino acids set forth in any of SEQ ID NOs. 32, 34, 36, 38 or 40; or a sequence of amino acids encoded by a sequence of nucleotides that hybridizes under conditions of high stringency along at least 70%, at least 80% or at least 90% of its length to a sequence of nucleotides that encodes a sequence of amino acids set forth in any of SEQ ID NOs. 32, 34, 36, 38 or 40. The fiber proteins can be modified, such as described herein, by replacement of the N-terminus to facilitate incorporation into the viral particle of a different subgroup, particularly subgroup C. Such modification is generally inclusion of at least 16 or 17 amino acids up to about 60 or 61 or more contiguous amino acids from the N-terminus of the native fiber such that dendritic cell targeting is introduced.

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In one exemplary embodiment, a packaging cell strategy is used to produce particles of a fiber-deleted Ad5 vector containing fiber proteins from Ads of subgroup B (Ad3, Ad16, Ad35), subgroup C (Ad5), and subgroup D (Ad19p, Ad30, Ad37). Nucleotide and amino acid sequences of Ad fibers are set forth in SEQ ID NOs. 41-44 (exemplary chimeric fibers) and 31-40 (exemplary wild type fibers that can be modified by replacement of the N-terminus). The resulting particles exhibit significant differences in dendritic cells tropism as demonstrated by their ability to infect primary murine bone marrow-derived DC *in vitro*. Furthermore, the particles pseudotyped with the subgroup D particles efficiently and specifically target dendritic cells. As described in the herein (see *e.g.*, the

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Examples) subgroup B fibers appear to bind to receptors distinct from and more ubiquitously expressed than those bound by subgroup D fibers.

While particles with the Ad5 fiber infect dendritic cells rather poorly, vector particles pseudotyped with subgroup D fibers or portions thereof, such as the Ad19p and Ad37, were particularly effective. Thus, adenovirus particles, particularly subgroup C particles, modified to express all or a portion of a subgroup D fiber, efficiently target dendritic cells and can be used to deliver heterologous nucleic acids to such cells *in vivo* and *ex vivo*. In addition, such particles have reduced binding to HSP-expressing cells, such as hepatocytes and to CAR-expressing cells compared to unmodified subgroup B viral particles.

b. Efficient Targeting

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Provided herein are recombinant adenoviruses with a limited tropism that target dendritic cells. The recombinant adenoviruses can be used for gene therapy and/or vaccination approaches. Administration can be effected *in vivo*, such as systemically, or *ex vivo* by contacting cells enriched for or containing dendritic cells.

The recombinant adenoviruses provided herein have a variety of advantageous properties. The particles provided herein more efficiently infect dendritic cells than Ad5 particles or Ad5 particles that express subgroup B fibers, and hence are more immunogenic following direct *in vivo* administration. The vectors provided herein that efficiently target dendritic cells permit not only *ex vivo* delivery, but direct *in vivo* administration, thereby eliminating the need for removal of cells, *ex vivo* cell culture, and infusion.

4. Additional Modifications

The modified adenoviruses provided herein not only exhibit

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improved tropism for dendritic cells, but also reduced binding to HSP, which is expressed on liver cells. The modified particles can be further modified to be detargeted from CAR, HSP, a_v integrin, or any other native receptors, by any of the capsid mutations described below or well known to those of skill in the art.

The vectors provided herein also can be modified by including a RGD peptide in the fiber protein. It has been shown (see, e.g., Okada et al. (Cancer Res. 61:7913-7919 (2001)) that incorporation of an RGD peptide into the fiber protein increased infection of a murine DC line approximately two-fold. The cell line/Ad system was then used to evaluate anti-tumor responses in a mouse tumor xenograft model. When DCs were infected ex vivo using equal particle numbers of the wild type or modified vectors and then re-infused into mice, the modified vector was able to stimulate a significantly better immune response against the model antigen.

The particles provided herein also can be further modified by inclusion of heterologous nucleic acid that provides a therapeutic product, and formulated for administration as vaccines. The adenovirus particles and vectors can deliver heterologous nucleic acids to dendritic cells to alter dendritic cell antigen presentation, cytockine production and other dendritic cell functions.

D. Adenovirus vector detargeting

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Described below are modifications of the viral capsid that ablate the interaction of an adenovirus with its natural receptors. In particular, fiber modifications that result in ablation of the interaction of an adenvirus with HSP are described. These fiber modifications can be combined with other capsid protein modifications, such as other fiber modifications and/or penton and/or hexon modifications, to fully ablate viral interactions with natural receptors, when expressed on a viral particle. The

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modification should not disrupt trimer formation or transport of fiber into the nucleus. The entire fiber of a serotype that binds to HSP can be replaced with all or a portion of a fiber that does not bind to HSP. Generally in such instances, the N-terminus of the replacing fiber is modified to resemble or to be identical to the replaced fiber to improve its incorporation into the viral particle. The number of amino acids at the N-terminus required can be empirically determined, but is typically between about 5-20, 10-17, 10-20, 10-50, 10-70, 10-100, amino acids, more amino acids can be included if convenient. The precise number also can be based upon the presence of convenient restriction sites in the encoding nucleic acid and other such considerations. Generally at least about 5-20, such as 16, 17, or 18, amino acids are required.

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The adenovirus fiber protein is a major determinant of adenovirus tropism (Gall *et al.* (1996) *J. Virol.* 70:2116-2123; Stevenson *et al.* (1995) *J. Virol.* 69:2850-2857). Dogma in the field has been that adenoviral entry occurs via binding to CAR and integrins. This is underscored by published data (Einfeld *et al.* (2001) *J. Virology* 75:11284-11291). The published are not the predominant ones that act *in vivo*. The dominant entry pathway for hepatocytes *in vivo* involves a mechanism mediated by the fiber shaft, such as Ad5 shaft, through heparin sulfate proteoglycans binding (see, published U.S. application Nos. 2004-0002060 and 2003-0215948).

Elimination of this binding eliminates entry via HSP binding, such as in hepatocytes. Adenoviral fiber shaft modifications that ablate viral interaction with HSP are described in the Examples below and in published U.S. application Nos. 2004-0002060 and 2003-0215948. Thus, efficient detargeting of adenovirus *in vivo* can be achieved with appropriately designed fiber proteins. Suitable modifications, such as described herein, can be made with respect to any adenovirus in which

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the wild-type interacts with HSP. The ability of an adenoviral vector to interact with HSP is modified by replacing the fiber protein or at least the binding portion thereof with a fiber (or corresponding portion thereof) that does not bind to HSP thereby reducing or eliminating binding to HSP.

This reduction or elimination of HSP binding can be manifested *in vivo* as reduced or eliminated transduction of liver cells in animals to whom the resulting viral particles are administered compared to the unmodifed particle. Modifications include insertions, deletions, individual amino acid mutations and other mutations that alter the structure of the fiber shaft such that the HSP binding of the modified fiber protein is ablated when compared to the HSP binding of the wild-type fiber protein.

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An adenoviral fiber protein is modified by mutating one or more of the amino acids that interact with HSP. For example, the HSP binding motif of the modified fiber protein is no longer able to interact with HSP on the cell surface, thus ablating the viral interaction with HSP. For example, the adenoviral fiber is from a subgroup C adenovirus. Binding to HSP can be eliminated or reduced by mutating the fiber shaft in order to modify the ability of the HSP binding motif, which is, for example, KKTK sequence (SEQ ID NO. 45) located between amino acid residues 91 to 94 in the Ad5 fiber (SEQ ID NO. 2), to interact with HSP. The fiber proteins are modified by chemical and biological techniques known to those skilled in the art, such as site directed mutagenesis of nucleic acid encoding the fiber or other techniques as illustrated herein.

In another aspect of this embodiment, the ability of a fiber to interact with HSP is modified by replacing the wild-type fiber shaft with a fiber shaft, or portion thereof, of an adenovirus that does not interact with HSP to produce chimeric fiber proteins. The portion is sufficient to reduce or eliminate interaction with HSP. Examples of adenoviruses having fiber shafts that do not interact with HSP include (a) adenoviruses

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of subgroup B, such as, but are not limited to, Ad3, Ad7, Ad11, Ad16, Ad21, Ad34 and Ad35 which do not have interaction with HSP, (b) adenoviruses of subgroup F, such as, but are not limited to, Ad40 and Ad41, specifically the short fiber, and (c) adenoviruses of subgroup D, such as but are not limited to, Ad19p, Ad30, Ad37 and Ad46.

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In another embodiment, adenoviral fiber shaft modifications and/or pseudotyped fibers that ablate viral interaction with HSP in combination with adenoviral fiber knob modifications that ablate viral interactions with CAR are provided. Suitable adenoviral fiber modifications include the 10 fiber knob modifications described in the Examples below and modifications known to those of skill in the art (see published U.S. application Nos. 2004-002060 and 2003-0215948; see, also, US. Patent Application Serial No. 09/870,203, filed on May 30, 2001, published as U.S. Published application No. 20020137213, and International Patent Application No. PCT/EP01/06286, filed on June 1, 2001, published as WO 01/92299). Modifications of the fiber include mutations of at least one amino acid in the CD loop of a wild-type fiber protein of an adenovirus from subgroup C (such as, e.g., Ad2 or Ad5), subgroup D (such as, e.g., Ad19p, Ad30 or Ad37), subgroup E, or the long wild-type 20 fiber of an adenovirus from subgroup F, whereby the ability of a fiber protein to bind to CAR is reduced or substantially eliminated. The fiber proteins with ablated CAR interaction are modified by chemical and biological techniques known to those skilled in the art and as described herein.

Alternatively, adenoviral fiber modifications are made by replacing the wild-type fiber knob with a fiber knob of an adenovirus that does not interact with CAR. The fiber protein also will be selected so that it does not interact with HSP. Examples of adenoviruses having fiber knobs that do not interact with CAR include (a) adenoviruses of subgroup B, e.g.,

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Ad3, Ad7, Ad11, Ad16, Ad21, Ad34, Ad35; and (b) adenoviruses of subgroup F, e.g., Ad40 and Ad41, specifically the short fiber.

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In another embodiment, adenoviral fiber shaft modifications and/or pseudotyped fibers that ablate viral interaction with HSP in combination with penton modifications that ablate viral interactions with $\alpha_{\rm v}$ integrins are provided. Suitable adenoviral penton modifications include the penton modifications, which are well known to those of skill in the art (see, *e.g.*, U.S. Patent No. 5,731,190; see, also Einfeld *et al.* (2001) *J. Virology* 75:11284-11291; and Bai *et al.* (1993) *J. Virology* 67:5198-5205).

For example, penton interaction with $\alpha_{\rm v}$ integrins can be ablated (reduced or eliminated) by substitution of the RGD tripeptide motif, required for $\alpha_{\rm v}$ interaction, in penton with a different tripeptide that does not interact with an $\alpha_{\rm v}$ integrin. The penton proteins with ablated $\alpha_{\rm v}$ integrin interactions are modified by chemical and biological techniques known to those skilled in the art (see, *e.g.*, described U.S. Patent No. 6,731,190 and as illustrated herein).

Also provided are adenoviral fiber shaft modifications or pseudotyped fibers that ablate viral interaction with HSP in combination with adenoviral fiber knob modifications that ablate viral interactions with CAR and with penton modifications that ablate viral interactions with $\alpha_{\rm v}$ integrins. These modifications are described above and prepared using chemical and biological techniques known to those skilled in the art and as illustrated herein.

Preparation of fibers modified to eliminate or reduce HSP interactions and fibers modified to alter interactions with other receptors and cell surface proteins, such as CAR and/or α_v integrin, also is described in the Examples below. The nucleic acid and/or amino acid sequences of exemplary modified fibers, whose construction are described below) are set forth as SEQ ID NOs. 3-30 as follows:

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SEQ ID NOs. 3 and 4 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5FKO1, where 5F refers to adenovirus 5 fiber, KO1 is an exemplary mutation of the CAR interaction site described herein;

SEQ ID NOs. 5 and 6 set forth the encoding nucleotide sequence and amino acid sequence of the modified ber designated 5FKO1RGD, which further includes an RGD ligand to demonstrate retargeting;

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SEQ ID NOs. 7 and 8 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5FKO12, where 5F refers to adenovirus 5 fiber, KO12 is another exemplary mutation of the CAR interaction site described herein;

SEQ ID NOs. 9 and 10 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5F S* nuc, where 5F refers to adenovirus 5 fiber, S* is an exemplary mutation of the shaft that alters binding to HSP;

SEQ ID NOs. 11 and 12 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5F S*RGD nuc, which further includes an RGD ligand;

SEQ ID NOs. 13 and 14 set forth the encoding nucleotide sequence and amino acid sequence of the modified ber designated 5FKO1S*, which contain the KO1 and S* mutations;

SEQ ID NOs. 15 and 16 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5FKO1S*RGD, which further includes an RGD ligand;

SEQ ID NOs. 17 and 18 set forth the encoding nucleotide sequence and amino acid sequence of a Ad35 fiber;

SEQ ID NOs. 19 and 20 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 35FRGD, which is 35F fiber with an RGD ligand;

SEQ ID NOs. 21 and 22 set forth the encoding nucleotide sequence and amino acid sequence of a Ad41 short fiber;

SEQ ID NOs. 23 and 24 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 41sFRGD, which is 41F short fiber with an RGD ligand;

SEQ ID NOs. 25 and 26 set forth the encoding nucleotide sequence and amino acid sequence of Ad5 penton;

SEQ ID NOs. 27 and 28 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5TS35H, which is a chimeric fiber in which an Ad5 fiber tail and shaft regions (5TS; amino acids 1 to 403) are connected to an Ad35 fiber head region (35H; amino acids 137 to 323) to form the 5TS35H chimera; and

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SEQ ID NOs. 29 and 30 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 35TS5H, which is a chimeric fiber in which an Ad35 fiber tail and shaft regions (35TS; amino acids 1 to 136) are connected to an Ad5 fiber head region (5H; amino acids 404 to 581) to form the 35TS5H chimera.

The modified fibers are displayed on virus particles by modifying the fiber protein and optionally additional proteins. This can be achieved by preparing adenoviral vectors that express the modified capsid proteins and produce particles with modified fibers, or by packaging adenoviral vectors, particularly those that do not encode one or more capsid proteins in appropriate packaging lines. Hence, as discussed in detail below, adenoviral vectors and viral particles with modified fibers that do not bind to HSP are provided.

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Retargeting detargeted fibers

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The viral particles that are detargeted as described above, can be retargeted to selected cells and/or tissues by inclusion of an appropriate targeting ligand in the capsid. The ligand can be included in any of the capsid proteins, such as fiber, hexon and penton. Loci for inclusion of nucleic acid encoding a targeting ligand is known to those of skill in the art for a variety of adenovirus serotypes; if necessary appropriate loci and other parameters can be empirically determined.

The ligand can be produced as a fusion by inclusion of the coding sequences in the nucleic acid encoding a capsid protein, or chemically conjugated, such as via ionic, covalent or other interactions, to the capsid or bound to the capsid (e.g., by Ab-ligand fusion, where Ab binds capsid protein; or by disulfide bonding or other crosslinking moieties or chemistries).

Thus, for example, a modified fiber nucleic acid also can include sequences of nucleotides that encode a targeting ligand to produce viral particles that include a targeting ligand in the capsid. Targeting ligand and methods for including such ligands in viral capids are well known. For example, inclusion of targeting ligands in fiber proteins is described in U.S. Patent Nos. 5,543,328 and 5,756,086 and in U.S. Patent Application Serial No. 09/870,203, published as U.S. Published application No. 20020137213, and International Patent Application No. PCT/EP01/06286, published as WO 01/92299. For different serotypes and strains of adenoviruses, loci for insertion of targeting ligands can be empirically determined. For different serotypes and strains, such loci can vary.

Because the adenovirus fiber has a trimeric structure, the ligand can be selected or designed to have a trimeric structure so that up to three molecules of the ligand are present for each mature fiber. Such

ligands can be incorporated into the fiber protein using methods known in the art (see, e.g., U.S. Patent No. 5,756,086). Instead of the fiber, the targeting ligand can be included in the penton or hexon proteins. Inclusion of targeting ligands in penton (see for example, in U.S. Patent Nos. 5,731,190 and 5,965,431) and in hexon (see for example, in U.S. Patent No. 5,965,541) is known.

In one exemplary embodiment, the ligand is included in a fiber protein, which is a fiber protein mutated as described herein. The targeting ligand can be included, for example, within the HI loop of the fiber protein. Any ligand that can fit in the HI loop and still provide a functional virus is contemplated herein. Such ligands can be as long as or longer than 80-100 amino acids (see, e.g., Belousova et al. (2002) J. Virol. 76:8621-8631). Such ligands are added by techniques known in the art (see, e.g., published International Patent Application publication No. WO 99/39734 and U.S. Patent Application No. 09/482,682). Other ligands can be be discovered through techniques known to those skilled in the art. Some non-limiting examples of these techniques include phage display libraries or by screening other types of libraries. Such ligands include any that target dendritic cells.

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Targeting ligands include any chemical moiety that preferentially directs an adenoviral particle to a desired cell type and/or tissue, such as a dendritic cell. The categories of such ligands include, but are not limited to, peptides, polypeptides, single chain antibodies, and multimeric proteins. Specific ligands include the TNF superfamily of ligands which include tumor necrosis factors (or TNF's) such as, for example, TNF α and TNF β , lymphotoxins (LT), such as LT- α and LT- β , Fas ligand which binds to Fas antigen; CD40 ligand, which binds to the CD40 receptor of B-lymphocytes; CD30 ligand, which binds to the CD30 receptor of neoplastic cells of Hodgkin's lymphoma; CD27 ligand, NGF ligand, and

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OX-40 ligand; transferrin, which binds to the transferrin receptor located on tumor cells, activated T -cells, and neural tissue cells; ApoB, which binds to the LDL receptor of liver cells; alpha-2-macroglobulin, which binds to the LRP receptor of liver cells; alpha-l acid glycoprotein, which binds to the asialoglycoprotein receptor of liver; mannose-containing 5 peptides, which bind to the mannose receptor of macrophages; sialyl-Lewis-X antigen-containing peptides, which bind to the ELAM-I receptor of activated endothelial cells; CD34 ligand, which binds to the CD34 receptor of hematopoietic progenitor cells; ICAM-I, which binds to 10 the LFA-I (CD11b/CD18) receptor of lymphocytes, or to the Mac-I (CD11a/CD18) receptor of macrophages; M-CSF, which binds to the c-fms receptor of spleen and bone marrow macrophages; circumsporozoite protein, which binds to hepatic Plasmodium falciparum receptor of liver cells; VLA-4, which binds to the VCAM-I receptor of activated 15 endothelial cells; HIV gp120 and Class II MHC antigen, which bind to the CD4 receptor of T -helper cells; the LDL receptor binding region of the apolipoprotein E (ApoE) molecule; colony stimulating factor, or CSF, which binds to the CSF receptor; insulin-like growth factors, such as IGF-I and IGF-II, which bind to the IGF-I and IGF-II receptors, respectively; 20 Interleukins 1 through 14, which bind to the Interleukin 1 through 14 receptors, respectively; the Fv antigen-binding domain of an immunoglobulin; gelatinase (MMP) inhibitor; bombesin, gastrin-releasing peptide; substance P; somatostatin; luteinizing hormone releasing hormone (LHRH); vasoactive peptide (VIP); gastrin; melanocyte 25 stimulating hormone (MSH); cyclic RGD peptide and any other ligand or cell surface protein-binding (or targeting) molecule. Such ligands can be

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advantageously employed with the Ad5 particles pseudotyped with subgroup D adenovirus fiber, such as, for example, Ad19p, Ad30 or Ad37 fiber.

E. Nucleic acids, adenoviral vectors and cells containing the nucleic acids and cells containing the vectors

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Also provided are polynucleotides that encode modified, including chimeric and/or heterologous, capsid proteins and that encode vectors for preparation of adenovirus that express modified capsid proteins provided herein. The sequences of the wild-type adenovirus proteins from many different adenovirus serotypes are well known in the art and are modified as described herein or by any suitable method.

Also provided are vectors including the polynucleotides provided herein. Such vectors include partial or complete adenoviral genomes and plasmids. Such vectors are constructed by techniques known to those skilled in the art and as illustrated herein. Also provided are adenoviral vectors modified by replacing whole fiber protein, or portions thereof, with the fiber proteins, or appropriate portions thereof, from an adenovirus of a different serotype that more efficiently targets dendritic cells. Adenoviruses that target dendritic cells can be identified by using the methods described herein. Their fiber-encoding genes can then be used to pseudotype viruses, such as Ad5 or Ad2 and infection and gene delivery of adenoviruses with the heterologous or chimeric fibers can be detected. Among the adenoviral vectors provided herein are those of subgroup C, which include Ad2 and Ad5, in which the nucleic acid encoding the fiber knob and a portion or all of the fiber shaft domain is replaced with nucleic acid encoding fiber or an appropriate portion thereof from a subgroup D adenovirus, such as Ad19p, Ad30 or Ad37.

Thus, adenoviral fiber modifications or substitutions can be made in viral particles by replacing the entire fiber protein, or a portion thereof,

with the fiber protein of an adenovirus that more efficiently binds to receptors on dendritic cells. Generally the heterologous adenovirus fiber is from a subgroup D adenovirus, such as Ad19p, Ad30 or Ad37. Adenoviral vectors of subgroup C, such as Ad2 and Ad5, having a replaced fiber knob are prepared using techniques well known in the art and as illustrated herein.

In particular, as exemplified herein, the nucleic acid and/or amino acid sequences of exemplary heterologous and/or modified fibers for dendritic cell targeting are set forth as SEQ ID NOs. 31-44 as follows:

SEQ ID NOs. 31 and 32 set forth the encoding nucleotide sequence and amino acid sequence of Ad37 fiber.

SEQ ID NOs. 33 and 34 set forth the encoding nucleotide sequence and amino acid sequence of Ad19p fiber.

SEQ ID NOs. 35 and 36 set forth the encoding nucleotide sequence and amino acid sequence of Ad30 fiber.

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SEQ ID NOs. 37 and 38 set forth the encoding nucleotide sequence and amino acid sequence of Ad16 fiber.

SEQ ID NOs. 39 and 40 set forth the encoding nucleotide sequence and amino acid sequence of Ad35 fiber.

SEQ ID NOs. 41 and 42 set forth the encoding nucleotide sequence and amino acid sequence of Ad5/Ad16 chimeric fiber. The chimeric fiber contains the N-terminal 17 amino acids from Ad5 and the remainder of the sequence is from Ad16.

SEQ ID NOs. 43 and 44 set forth the encoding nucleotide sequence and amino acid sequence of Ad5/Ad35 chimeric fiber. The chimeric fiber contains the N-terminal 17 amino acids from Ad5 and the remainder of the sequence is from Ad35.

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1. Preparation of viral particles

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The packaging cells used to produce the viruses provided herein contain the nucleic acid encoding the capsid (i.e. fiber, penton, hexon) protein. Such nucleic acid can be transfected into the cell, generally as part of a plasmid, or it can be infected into the cell with a viral vector. It can be stably incorporated into the genome of the cell, thus providing for a stable cell line. Alternatively, nucleic acid encoding the heterologous or mutated capsid protein can be removed from the genome, in which case a transient complementing cell is employed.

The adenovirus genome to be packaged is transferred into the complementing cell by techniques known to those skilled in the art.

These techniques include transfection or infection with the adenovirus.

The nucleic acid encoding the mutated or heterologous fiber protein can be in this genome instead of in the packaging cell.

In certain cases, when the nucleic acid in the genome to be packaged encodes a mutated or heterologous fiber protein, it can be desirable for the packaging cell to also encode a fiber protein. Such protein can assist in the maturation and packaging of an infectious particle. Such protein can be a wild-type fiber protein or one modified such that it is unable to attach to the penton base protein and is for use, for example, in producer cells where the fiber is included to provide the packaging function and the vector encodes a full-length fiber.

The packaging cells are cultured under conditions that permit the production of the desired viral particle. The viral particles are recovered by standard techniques. An exemplary method for producing adenoviral particles provided herein is as follows. The nucleic acid encoding the mutated or heterologous capsid protein is made using standard techniques in an adenoviral shuttle plasmid. This plasmid contains the right end of the virus, in particular from the end of the E3 region through the right ITR.

This plasmid is co-transfected into competent cells of an *E. coli* strain, such as the well known *E. coli* strain BJ5183 (see, *e.g.*, Degryse (1996) *Gene 170*:45-50) along with a plasmid, which contains the remaining portion of the adenovirus genome, except for the E1 region and sometimes also the E2a region and also contains a corresponding region of homology. Homologous recombination between the two plasmids generates a full-length plasmid encoding the entire adenoviral vector genome.

This full-length adenoviral vector genome plasmid is then transfected into a complementing cell line. The transfection can be performed in the presence of a reagent that directs adenoviral particle entry into producer cells. Such reagents include, but are not limited to, polycations and bifunctional reagents, such as those described herein. A complementing cell, for example, is a cell of the PER.C6 cell line, which contains the adenoviral E1 gene (PER.C6 is available, for example, from Crucell, The Netherlands; deposited under ECACC accession no. 96022940; see, also Fallaux *et al.* (1998) *Hum. Gene Ther.* 9:1909-1907; see, also, U.S. Patent No. 5,994,128) or an AE1-2a cell (see, Gorziglia *et al.* (1996) *J. Virology* 70:4173-4178; and Von Seggern *et al.* (1998) *J. Gen. Virol.* 79:1461-1468)).

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AE1-2a cells are derivatives of the A549 lung carcinoma line (ATCC # CCL 185) with chromosomal insertions of the plasmids pGRE5-2.E1 (also referred to as GRE5-E1-SV40-Hygro construct and listed in SEQ ID NO. 47) and pMNeoE2a-3.1 (also referred to as MMTV-E2a-SV40-Neo construct and listed in SEQ ID NO. 48), which provide complementation of the adenoviral E1 and E2a functions, respectively.

The 633 cell line (see, von Seggern *et al.* (2000) *J. Virology* 74:354-362), which stably expresses the adenovirus serotype 5 wild-type fiber protein, and was derived from the AE1-2a cell line, is another

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example of complementing cells. When the cell line is 633 cell line, the final passage of the adenoviral vector is performed on another complementing cell line (e.g., Per.C6), which does not express wild-type Ad5 fiber.

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The transfected complementing cells are maintained under standard cell culture conditions. The adenoviral plasmids recombine to form the adenoviral genome that is packaged. The particles are infectious, but replication deficient because their genome is missing at least the E1 genes. When performed in the 633 cells the particles contain wild-type and mutated or heterologous fiber proteins. They are recovered from the crude viral lysate, amplified, and are purified by standard techniques.

The recovered particles can be used to infect PER.C6 or AE1-2a cells. This permits the recovery of particles whose capsids contain only the desired mutated fiber. This two-step procedure provides high titer batches of the adenoviral particles provided herein. The adenoviral particles can be replication competent or replication incompetent.

In one embodiment, the particles selectively replicate in certain predetermined target tissue but are replication incompetent in other cells and tissues. In a particular embodiment, the adenoviral particles replicate in abnormally proliferating tissue, such as solid tumors and other neoplasms. In replication conditional adenoviruses, a gene essential for replication is placed under control of a heterologous promoter which is cell or tissue specific. For example, the E1a gene is placed under control of a promoter which is active in a tumor cell to produce an oncolytic adenovirus or oncolytic adenoviral vector. Administration of oncolytic adenoviral vectors to tumor cells kills the tumor cells. Such replication conditional adenoviral particles and vectors can be produced by techniques known to those skilled in the art, such as those disclosed in the above-referenced U.S. Patent Nos. 5,998,205 and 5,801,029. These

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particles and vectors can be produced in adenoviral packaging cells as disclosed above. Generally packaging cells are those that have been designed to limit homologous recombination that could lead to wild-type adenoviral particles. Such cells are well known and include the packaging cell known as PER.C6 (see, *e.g.*, U.S. Patent Nos. 5,994,128 and 6,033,908; deposited under ECACC accession no. 96022940).

2. Adenoviral vectors and particles

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The adenovirus as used herein for production of the adenoviral vectors and particles can be of any serotype, such as an Ad5 or Ad2. Adenoviral stocks that can be employed as a source of adenovirus or 10 adenoviral coat protein, such as fiber and/or penton base, can be amplified from the adenoviral serotypes 1 through 51, which are currently available from the American Type Culture Collection (ATCC, Rockville, Md.), or from any other serotype of adenovirus available from any other source. For instance, an adenovirus can be of subgroup A (e.g., 15 serotypes 12, 18, 31), subgroup B (e.g., serotypes 3, 7, 11, 14, 16, 21, 34, 35, 50), subgroup C (e.g., serotypes 1, 2, 5, 6), subgroup D (e.g., serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51), subgroup E (serotype 4), subgroup F (serotype 40, 41), or any other adenoviral serotype. In certain embodiments, the adenovirus is a 20 subgroup C adenovirus. Subgroup C adenoviruses which are modified in as described herein, include, but are not limited to, Ad2 and Ad5.

The adenoviral vectors provided herein can be used to study cell transduction and gene expression *in vitro* or in various animal models.

25 The latter case includes *ex vivo* techniques, in which cells are transduced *in vitro* and then administered to the animal. They also can be used to conduct gene therapy on humans or other animals. Such gene therapy can be *ex vivo* or *in vivo*. For *in vivo* gene therapy, the adenoviral particles in a pharmaceutically-acceptable carrier are delivered to a human

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in a therapeutically effective amount in order to prevent, treat, or ameliorate a disease or other medical condition in the human through the introduction of a heterologous gene that encodes a therapeutic protein into cells in such human. The adenoviruses are delivered at a dose ranging from approximately 1 particle per kilogram of body weight to approximately 10¹⁴ particles per kilogram of body weight. Generally, they are delivered at a dose of approximately 10⁶ particles per kilogram of body weight to approximately 10¹³ particles per kilogram of body weight, and typically the dose ranges from approximately 10⁸ particles per kilogram of body weight to approximately 10¹² particles per kilogram of body weight.

Any vectors known to those of skill in the art can be employed and used to produce viral particles that include fibers modified to enhance binding and infectivity of dendritic cells.

a. Gutless vectors

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Gutted adenovirus vectors are those from which most or all viral genes have been deleted. They are grown by co-infection of the producing cells with a "helper" virus (such as using an El-deleted Ad vector), where the packaging cells express the E1 gene products. The helper virus trans-complements the missing Ad functions, including production of the viral structural proteins needed for particle assembly. To incorporate the capsid modifications into a gutted adenoviral vector capsid, the changes must be made to the helper virus as described herein. All the necessary Ad proteins including the modified capsid protein are provided by the modified helper virus, and the gutted adenovirus particles are equipped with the particular modified capsid expressed by the host cells. The E1a, Eb, E2a, E2b and E4 are generally required for viral replication and packaging. If these genes are deleted, then the packaging cell must provide these genes or functional equivalents.

A helper adenovirus vector genome and a gutless adenoviral vector genome are delivered to packaging cells. The cells are maintained under standard cell maintenance or growth conditions, whereby the helper vector genome and the packaging cell together provide the complementing proteins for the packaging of the adenoviral vector particle. Such gutless adenoviral vector particles are recovered by standard techniques. The helper vector genome can be delivered in the form of a plasmid or similar construct by standard transfection techniques, or it can be delivered through infection by a viral particle containing the genome. Such viral particle is commonly called a helper virus. Similarly, the gutless adenoviral vector genome can be delivered to the cell by transfection or viral infection.

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The helper virus genome can be the modified adenovirus vector genome as disclosed herein. Such genome also can be prepared or designed so that it lacks the genes encoding the adenovirus E1A and E1B proteins. In addition, the genome can further lack the adenovirus genes encoding the adenovirus E3 proteins. Alternatively, the genes encoding such proteins can be present but mutated so that they do not encode functional E1A, E1B and E3 proteins. Furthermore, such vector genome can not encode other functional early proteins, such as E2A, E2B3, and E4 proteins. Alternatively, the genes encoding such other early proteins can be present but mutated so that they do not encode functional proteins.

In producing the gutless vectors, the helper virus genome also is packaged, thereby producing helper virus. In order to minimize the amount of helper virus produced and maximize the amount of gutless vector particles produced, the packaging sequence in the helper virus genome can be deleted or otherwise modified so that packaging of the helper virus genome is prevented or limited. Since the gutless vector

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genome will have an unmodified packaging sequence, it will be preferentially packaged.

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One way to do this is to mutate the packaging sequence by deleting one or more of the nucleotides comprising the sequence or otherwise mutating the sequence to inactivate or hamper the packaging function. One exemplary approach is to engineer the helper genome so that recombinase target sites flank the packaging sequence and to provide a recombinase in the packaging cell. The action of recombinase on such sites results in the removal of the packaging sequence from the helper virus genome. The recombinase can be provided by a nucleotide sequence in the packaging cell that encodes the recombinase. Such sequence can be stably integrated into the genome of the packaging cell. Various kinds of recombinase are known by those skilled in the art, and include, but are not limited to, Cre recombinase, which operates on so-called lox sites, which are engineered on either side of the packaging sequence as discussed above (see, e.g., U.S. Patent Nos. 5,919, 676, 6,080,569 and 5,919,676; see, also, e.g., Morsy and Caskey, Molecular Medicine Today, Jan. 1999, pgs. 18-24).

An example of a gutless vector is pAdARSVDys (Haecker *et al.* (1996) *Hum Gene Ther.* 7:1907-1914)). This plasmid contains a full-length human dystrophin cDNA driven by the RSV promoter and flanked by Ad inverted terminal repeats and packaging signals. 293 cells are infected with a first-generation Ad, which serves as a helper virus, and then transfected with purified pAdARSVDys DNA. The helper Ad genome and the pAdARSVDys DNA are replicated as Ad chromosomes, and packaged into particles using the viral proteins produced by the helper virus. Particles are isolated and the pAdARSVDys-containing particles separated from the helper by virtue of their smaller genome size and therefore different density on CsCl gradients. Other examples of

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gutless adenoviral vectors are known (see, e.g., Sandig et al. (2000) Proc. Natl. Acad. Sci. U.S.A. 97(3):1002-7).

b. Oncolytic vectors

Oncolytic adenoviruses are viruses that replicate selectively in tumor cells. Such vectors generally will not be useful for targeting dendritic cells, unless such cells are malignant. Briefly, oncolytic vectors are designed to amplify the input virus dose due to viral replication in the tumor, leading to spread of the virus throughout the tumor mass. In situ replication of adenoviruses leads to cell lysis. This in situ replication 10 permits relatively low, non-toxic doses to be highly effective in the selective elimination of tumor cells. One approach to achieving selectivity is to introduce loss-of-function mutations in viral genes that are essential for growth in non-target cells but not in tumor cells (see, e.g., U.S. Patent No. 5,801,029). This strategy is exemplified by the use of Addl1520, 15 which has a deletion in the E1b-55KD gene. In normal cells, the adenoviral E1b-55KD protein is needed to bind to p53 to prevent apoptosis. In p53-deficient tumor cells, E1b-55K binding to p53 is unnecessary. Thus, deletion of E1b-55KD should restrict vector replication to p53-deficient tumor cells.

Another approach is to use tumor-selective promoters to control the expression of early viral genes required for replication (see, e.g., International PCT application Nos. WO 96/17053 and WO 99/25860). Thus, in this approach the adenoviruses selectively replicate and lyse tumor cells if the gene that is essential for replication is under the control of a promoter or other transcriptional regulatory element that is tumor-selective.

For example oncolytic adenoviral vectors that contain a cancer selective regulatory region operatively linked to an adenoviral gene essential for adenoviral replication are known (see, e.g., U.S. Patent No.

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5,998,205). Adenoviral genes essential for replication include, but are not limited to, E1a, E1b, E2a, E2b and E4. For example, an exemplary oncolytic adenoviral vector has a cancer selective regulatory region operatively linked to the E1a gene. In other embodiments, the oncolytic adenoviral vector has a cancer selective regulatory region of the present invention operatively linked to the E1a gene and a second cancer selective regulatory region operatively linked to the E4 gene. The vectors also can include at least one therapeutic transgene, such as, but not limited to, a polynucleotide encoding a cytokine such as GM-CSF that can stimulate a systemic immune response against tumor cells.

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Other exemplary oncolytic adenoviral vectors include those in which expression of an adenoviral gene, which is essential for replication, is controlled by E2F-responsive promoters, which are selectively transactivated in cancer cells. Thus, vectors that contain an adenoviral nucleic acid backbone that contain in sequential order: A left ITR, an adenoviral packaging signal, a termination signal sequence, an E2F responsive promoter which is operably linked to a first gene, such as E1a, essential for replication of the recombinant viral vector and a right ITR (see, published International PCT application No. WO02/06786, and U.S. Patent No. 5,998,205).

In other embodiments, the oncolytic adenoviral vector has a cancer selective regulatory region operatively linked to the E1a gene and a second cancer selective regulatory region operatively linked to the E4 gene. The vectors also can carry at least one therapeutic transgene, such as, but not limited to, a polynucleotide encoding a cytokine such as GM-CSF that can stimulate a systemic immune response against tumor cells.

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3. Packaging

The viral particles provided herein can be made by any method known to those of skill in the art. Generally they are prepared by growing the adenovirus vector that contains nucleic acid that encodes the modified or heterologous capsid protein in standard adenovirus packaging cells to produce particles that express the modified or heterologous capsid proteins. Alternatively, the vectors do not encode fiber proteins. Such vectors are packaged in producer cells to produce particles that express the modified fiber proteins.

10 As discussed, recombinant adenoviral vectors generally have at least a deletion in the first viral early gene region, referred to as E1, which includes the E1a and E1b regions. Deletion of the viral E1 region renders the recombinant adenovirus defective for replication and incapable of producing infectious viral particles in subsequently infected target cells.

Thus, to enable E1-deleted adenovirus genome replication and to produce virus particles requires a system of complementation which provides the missing E1 gene product. E1 complementation is typically provided by a cell line expressing E1, such as the human embryonic kidney packaging cell line, i.e. an epithelial cell line, called 293. Cell line 293 contains the

20 E1 region of adenovirus, which provides E1 gene region products to "support" the growth of E1-deleted virus in the cell line (see, e.g., Graham et al., J. Gen. Virol. 36: 59-71, 1977). Additionally, cell lines that may be usable for production of defective adenovirus having a portion of the adenovirus E4 region have been reported (WO 96/22378).

25 Multiply deficient adenoviral vectors and complementing cell lines have also been described (WO 95/34671, U.S. Patent No. 5,994,106).

For example, copending U.S. application Serial No. 09/482,682 (also filed as International PCT application No. PCT/EP00/00265, filed January 14, 200, published as International PCT application No.

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WO/0042208) provides packaging cell lines that support viral vectors with deletions of major portions of the viral genome, without the need for helper viruses and also provides cell lines and helper viruses for use with helper-dependent vectors. The packaging cell line has heterologous DNA stably integrated into the chromosomes of the cellular genome. The heterologous DNA sequence encodes one or more adenovirus regulatory and/or structural polypeptides that complement the genes deleted or mutated in the adenovirus vector genome to be replicated and packaged.

Packaging cell lines express, for example, one or more adenovirus structural proteins, polypeptides, or fragments thereof, such as penton base, hexon, fiber, polypeptide Illa, polypeptide V, polypeptide VI, polypeptide VIII, and biologically active fragments thereof. The expression can be constitutive or under the control of a regulatable promoter. These cell lines are particularly designed for expression of recombinant adenoviruses intended for delivery of therapeutic products. For use herein, such packaging cell lines can express the modified or heterologous capsid proteins, such as the fiber proteins whose binding and infection of dendritic cells is enhanced.

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Particular packaging cell lines complement viral vectors having a deletion or mutation of a DNA sequence encoding an adenovirus structural protein, regulatory polypeptides E1A and E1B, and/or one or more of the following regulatory proteins or polypeptides: E2A, E2B, E3, E4, L4, or fragments thereof.

The packaging cell lines are produced by introducing each DNA molecule into the cells and then into the genome via a separate complementing plasmid or plurality of DNA molecules encoding the complementing proteins can be introduced via a single complementing plasmid. Of interest herein, is a variation in which the complementing plasmid includes DNA encoding adenovirus fiber protein (or a chimeric or

modified variant thereof), from Ad virus of subgroup D, such as Ad19p or Ad37.

For applications, such as therapeutic applications, the delivery plasmid further can include a nucleotide sequence encoding a heterologous polypeptide. Exemplary delivery plasmids include, but are not limited to, pDV44, p Δ E1B β -gal and p Δ E1sp1B (Microbix Biosystems; see also, U.S. Patent No. 6,140,087 and U.S. Patent No. 6,379,943). In a similar or analogous manner, therapeutic nucleic acids, such as nucleic acids that encode therapeutic genes, can be introduced.

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The cell further includes a complementing plasmid encoding a fiber or other capsid protein as contemplated herein; the plasmid or portion thereof is integrated into a chromosome(s) of the cellular genome of the cell.

Typically, the packaging cell lines will contain nucleic acid encoding the capsid protein or modified capsid protein stably integrated into a chromosome or chromosomes in the cellular genome. The packaging cell line can be derived from a procaryotic cell line or from a eukaryotic cell line. While various embodiments suggest the use of mammalian cells, and more particularly, epithelial cell lines, a variety of other, non-epithelial cell lines are used in various embodiments. Thus, while various embodiments disclose the use of a cell line selected from among the 293, A549, W162, HeLa, Vero, 211, and 211A cell lines, any other cell lines suitable for such use are likewise contemplated herein.

4. Propagation and Scale-up of doubly-ablated adenoviral vectors

Since doubly ablated adenoviral vectors containing mutations in the fiber and/or penton capsid proteins result in inefficient cell binding and entry via the $CAR/\alpha v$ integrin entry pathway, scaled up technologies improve the growth and propagation of such vectors to produce high

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titers of the adenoviral vectors for clinical use. Thus, also provided is a method for scaling up the production of detargeted adenoviral vectors. The detargeted adenoviral vectors comprise an adenoviral vector modified to ablate the interaction of said vector with at least one host cell receptor compared with a wild-type adenoviral vector. The detargeted adenoviral vectors can comprise an adenoviral vector modified to ablate the interaction of said vector with one, two, three or more host cell receptors. Thus, the method is suitable for producing the detargeted adenoviral vectors disclosed herein.

As noted, growth and propagation of doubly and fully ablated adenoviral vectors is enhanced by new scale up technologies. Doubly ablated vectors contain mutations in the fiber and penton capsid proteins that result in inefficient cell binding and entry via the normal cellular entry pathway using CAR and integrins. These vectors are fully detargeted *in vitro* and, thus, alternative cellular entry strategies allow for the efficient growth and generation of high titer preparations.

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Two strategies have been envisioned to scale up vectors that are detargeted via fiber and/or penton modifications. These include: (a) the use of pseudoreceptor cell lines engineered to express a surface receptor that binds a ligand displayed on the vector (see, e.g., International PCT application No. WO 98/54346) and (b) complementing cell lines that are engineered to express native fiber and that can be engineered to express native fiber and penton (see, e.g., International PCT application No. WO 00/42208). Although these systems have shown promise for scaling up ablated adenoviral vectors, there is a need to develop a system for the simple, efficient production of the fully detargeted adenoviral vector for therapeutic uses.

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Provided herein is a scale-up method for the propagation of detargeted adenoviral vectors. The method uses polycations and/or bifunctional reagents, which when added to tissue culture medium, bind adenoviral particles and direct their entry into the producer cells.

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Reagents (also called medium additives) also can be included in the tissue culture medium containing producer cells to be infected with the detargeted adenoviral vectors. Alternatively the reagents can be premixed with the virus, which mixture is then added to the tissue producer cells. The reagents can be added to tissue culture medium containing producer cells, or producer cells can be added to tissue culture medium containing the reagents. Any suitable producer cell known to the skilled artisan can be used in the present methods. The reagents can be added at the same time that the producer cells are infected with detargeted adenoviral vectors. Generally the reagents are present in the tissue culture medium prior to infection by the detargeted adenoviral vectors. The medium additives are maintained in the tissue culture medium during vector growth, spread and propagation. High titer yields of adenoviral vectors are obtained by this method.

Reagents which are useful in this method are those that are
capable of directing adenoviral particle entry into the producer cells. Such reagents include, but are not limited to, polycations and bifunctional reagents. Suitable polycations include, but are not limited to, polytheylenimine; protamine sulfate; poly-L-lysine hydrobromide; poly(dimethyl diallyl ammonium) chloride (Merquat(r)-100, Merquat(r)280,
Merquat(r)550); poly-L-arginine hydrochloride; poly-L-histidine; poly(4-vinylpyridine), poly(4-vinylpyridine) hydrochloride; poly(4-vinylpyridine) pyridine-co-styrene); poly(4-vinylpyridinium poly(hydrogen fluoride)); poly(4-vinylpyridinium-P-toluene sulfonate); poly(4-vinylpyridinium-tribro-

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mide); poly(4-vinylpyrrolidone-co-2-dimethylamino-ethyl methacrylate); polyvinylpyrrolidone, cross-linked; poly vinylpyrrolidone, poly(melamine-co-formaldehyde); partially methylated; hexadimethrine bromide; poly(Glu, Lys) 1:4 hydrobromide; poly(Lys, Ala) 3:1 hydrobromide; poly(Lys, Ala) 2:1 hydrobromide; poly-L-lysine succinylated; poly(Lys, Ala) 1:1 hydrobromide; and poly(Lys, Trp) 1:4 hydrobromide.

Suitable bifunctional reagents include, but are not limited to, antibodies or peptides that bind to the adenoviral capsid and that also contain a ligand that allows interaction with specific cell surface receptors of the producer cells. Examples of bifunctional reagents include: (a) anti-fiber antibody ligand fusions, (b) anti-fiber-Fab-FGF conjugate, (c) anti-penton-antibody ligand fusions, (d) anti-hexon antibody ligand fusions and (e) polylysine-peptide fusions. The ligand is any ligand that will bind to any cell surface receptor found on the producer cells.

15 F. Adenovirus Expression Vector Systems

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The adenovirus vector genome that is encapsulated in the virus particle and that expresses exogenous genes in a gene therapy setting is provided. The components of an recombinant adenovirus vector genome include the ability to express selected adenovirus structural genes, to express a desired exogenous protein, and to contain sufficient replication and packaging signals that the genome is packaged into a gene delivery vector particle. An exemplary replication signal is an adenovirus inverted terminal repeat containing an adenovirus origin of replication, as is well known and described herein. Although adenovirus include many proteins, not all adenovirus proteins are required for assembly of a recombinant adenovirus particle (vector). Thus, deletion of the appropriate genes from a recombinant Ad vector permits accommodation of even larger "foreign" DNA segments.

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One recombinant adenovirus vector genome is "helper independent" so that genome can replicate and be packaged without the help of a second, complementing helper virus. Complementation is provided by a packaging cell. Particuarly contemplated are helper dependent systems. In an exemplary embodiment, the adenovirus vector genome does not encode a functional adenovirus fiber protein. A nonfunctional fiber gene refers to a deletion, mutation or other modification to the adenovirus fiber gene such that the gene does not express any or insufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of the fiber gene by a complementing plasmid or packaging cell line. Such a genome is referred to as a "fiberless" genome, not to be confused with a fiberless particle. Alternatively, a fiber protein may be encoded but is insufficiently expressed to result in a fiber containing particle.

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Thus, contemplated for use are helper-independent fiberless recombinant adenovirus vector genomes that include genes that (a) epxress all adenovirus structural gene products but express insufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene, (b) express an exogenous protein, and (c) contains an adenovirus packaging signal and inverted terminal repeats containing adenovirus origin of replication.

The adenovirus vector genome is propagated *in vitro* in the form of rDNA plasmids containing the genome, and upon introduction into an appropriate host, the viral genetic elements provide for viral genome replication and packaging rather than plasmid-based propagation. Exemplary methods for preparing an Ad-vector genome are described in the Examples.

A vector herein includes a nucleic acid (such as DNA) molecule capable of autonomous replication in a cell and to which a DNA segment,

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e.g., a gene or polynucleotide, can be operatively linked to bring about replication of the attached segment. For purposes herein, one of the nucleotide segments to be operatively linked to vector sequences encodes at least a portion of a therapeutic nucleic acid molecule. As noted above, therapeutic nucleic acid molecules include those encoding proteins and also those that encode regulatory factors that can lead to expression or inhibition or alteration of expression of a gene product in a dendritic cell.

1. Nucleic Acid Gene Expression Cassettes

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In various embodiments, a peptide-coding sequence of the therapeutic gene is inserted into an expression vector and expressed; however, it also is feasible to construct an expression vector which also includes some non-coding sequences as well. Generally, however, non-coding sequences are excluded. Alternatively, a nucleotide sequence for a soluble form of a polypeptide may be utilized. Another therapeutic viral vector includes a nucleotide sequence encoding at least a portion of a therapeutic nucleotide sequence operatively linked to the expression vector for expression of the coding sequence in the therapeutic nucleotide sequence.

The choice of viral vector into which a therapeutic nucleic acid molecule is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., vector replication and protein expression, and the host cell to be transformed -- these being limitations inherent in the art of constructing recombinant DNA molecules. Although certain adenovirus serotypes are recited herein in the form of specific examples, it should be understood that the use of any adenovirus serotype, including hybrids and derivatives thereof are contemplated. Of particular interest, is the use of fiber that targets the resulting viral particle to dendritic cells.

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2. Promoters

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As noted elsewhere herein, an expression nucleic acid in an Adderived vector also include a promoter, particularly a tissue or cell specific promoter, such as one expressed dendritic cells. Promoters are nucleic acid fragments that contain a DNA sequence that controls the expression of a gene located 3' or downstream of the promoter. The promoter is the DNA sequence to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene, typically located 3' of the promoter. A promoter also includes DNA sequences which direct the initiation of transcription, including those to which RNA polymerase specifically binds. If more than one nucleic acid sequence encoding a particular polypeptide or protein is included in a therapeutic viral vector or nucleotide sequence, more than one promoter or enhancer element may be included, particularly if that would enhance efficiency of expression. Regulatable (inducible) as well as constitutive promoters may be used, either on separate vectors or on the same vector. For example, some useful regulatable promoters are those of the CREB-regulated gene family and include inhibin, gonadotropin, cytochrome c, glucagon and other. (See, e.g., International PCT application No. WO 96/14061). The promoter selected can be selected from a dendritic cell-specific gene, such as $NF\kappa B$.

A regulatable or inducible promoter is a promoter where the rate of RNA polymerase binding and initiation is modulated by external stimuli. (see, e.g., U.S. Patent Nos. 5,750,396 and 5,998,205). Such stimuli include various compounds or compositions, light, heat, stress, chemical energy sources, and the like. Inducible, suppressible and repressible promoters are considered regulatable promoters. Regulatable promoters also can include tissue-specific promoters. Tissue-specific promoters direct the expression of the gene to which they are operably linked to a

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specific cell type. Tissue-specific promoters cause the gene located 3' of it to be expressed predominantly, if not exclusively, in the specific cells where the promoter expressed its endogenous gene. Typically, it appears that if a tissue-specific promoter expresses the gene located 3' of it at all, then it is expressed appropriately in the correct cell types (see, *e.g.*, Palmiter et al. (1986) Ann. Rev. Genet. 20: 465-499).

G. Heterologous Polynucleotides and Therapeutic Nucleic Acids

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The packaged adenoviral genome also can contain a heterologous polynucleotide that encodes a product of interest, such as a therapeutic protein. Adenoviral genomes containing heterologous polynucleotides are well known (see, e.g., U.S. Patent Nos. 5,998,205, 6,156,497, 5,935,935, and 5,801,029). These can be used for *in vitro* and *in vivo* delivery of the products of heterologous polynucleotides or the heterologous polynucleotides.

The adenoviral particles provided herein can be used to engineer a cell to express a protein that it otherwise does not express or does not express in sufficient quantities. This genetic engineering is accomplished by infecting the desired cell with an adenoviral particle whose genome includes a desired heterologous polynucleotide. The heterologous polynucleotide is then expressed in the genetically engineered cells. For use herein the cell is generally a mammalian cell, and is typically a primate cell, including a human cell. The cell can be inside the body of the animal (*in vivo*) or outside the body (*in vitro*). Heterologous polynucleotides (also referred to as heterologous nucleic acid sequences) are included in the adenoviral genome within the particle and are added to that genome by techniques known in the art. Any heterologous polynucleotide of interest can be added, such as those disclosed in U.S. Patent No. 5,998,205, incorporated herein by reference.

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Polynucleotides that are introduced into an Ad genome or vector can be any that encode a protein of interest or that are regulatory sequences. In particular, the genomes can include heterologous nucleic acid encoding a product for expression in a dendritic cell for presentation or to alter the activity of the dendritic cell. For purposes herein, proteins include, but are not limited to tumor antigens. Tumor antigens included, but are not limited to carcinoembryonic antigen, NY-BR1, NY-ESO-1, MAGE-1, MAGE-3, BAGE, GAGE, SCP-1, SSX-1, SSX-2, SSX-4, CT-7, Her2/Neu, NY-BR-62, NY-BR-85 and tumor protein D52 (Scanlan and Jäger (2001) *Breast Cancer Res. 3*:95-98; Yu and Restifo (2002) *J. Clin. Invest.* 110:289-94). The following Table includes an exemplary list of tumor antigens and tissues expressing such antigens.

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| Antigen | Tissue |
|-----------|---|
| Oncofetal | |
| ОРА | Fetal pancreas |
| CEA | Colon, Rectal, Stomach, Lung, Pancreas, Kidney, Bladder, Head & Neck, Cervical, endometrial, ovarian, Breast |
| POA | Fetal pancreas |
| FAP | Fetal pancreas |
| PA8-15 | Pancreatic cancer cell line SUIT-2 |
| Adult | |
| CA 50 | Colorectal carcinoma cell line |
| CA 19-9 | Colon carcinoma cell line SW1116 |
| CA 242 | Colorectal carcinoma cell line COLO 205 |
| CAR-3 | Epidermoid carcinoma cell line A 431 |

| Antigen | Tissue |
|---|--|
| DU-PAN-2 | Pancreatic carcinoma cell line HPAF |
| Ypan-1 | Pancreatic carcinoma cell line SW1990 |
| Span-1 | п |
| BW494 | Pancreatic tumor tissue |
| MUSE 11 | Gastric cancer ascites fluid |
| L _{A1} | Embryonal carcinoma cells |
| Le ^a Fuc-L _{A1} | Colon adenocarcinoma Pancreatic adenocarcinoma |
| Le ^b . | Colon adenocarcinoma Pancreatic adenocarcinoma |
| 3-isoL _{M1} | Small cell lung carcinoma Glioma Medulloblastoma Teratocarcinoma cells |
| 3',6'-isoL _{D1} | Liver metastasis of colon cancer Embryonal carcinoma cells |
| Fuc-3'- isoL _{M1} Sialylated Le ^a | Gastrointestinal cancer |
| Fuc-3',6'- isoL _{D1} Disialylated Le ^a | Human colon adenocarcinoma |
| nL _{A1} i-Antigen | Colon cancer Lung cancer |

| Antigen | Tissue |
|---|---|
| SSEA-1 Le ^x Fuc-nL _{A1} | Teratocarcinoma Colon cancer |
| Dimeric Le ^x | Adenocarcinoma Colon cancer Liver cancer |
| Le ^v | Gastric cancer Breast cancer Colon cancer |
| 6'-L _{M1} | Colorectal carcinoma Lung carcinomas Primary hepatoma |
| Sialylated Le ^x or Fuc-3'-L _{M1} | Gastrointestinal cancer Lung carcinoma |
| | Gastric colon lung breast renal cancers |
| GB3 Globo-H | Burkitt's lymphoma breast cancer |
| Sulfatide | Mucinous cystadenocarcinoma, |
| Disulfated G _{A1} | Hepatocellular carcinoma |
| N-Glycolylneuraminic acid | Colon cancer |
| N-Glycolyl-G _{M2} | N-Glycolyl-G _{M2} |
| G _{M2} | Melanoma |
| OFA-I-1 OFA-I-2 | |
| | Glioma |
| | Germ cell tumors |
| G _{D2} | Melanoma |
| | Neuroblastoma |

| | Antigen | Tissue |
|----|---|------------------------------|
| | | Small cell lung carninoma |
| | | Glioma |
| 5 | G _{M3} Ag FCM1 2-39 IF43 gp-100 melanoma- associated antigen | Melanoma , |
| 10 | G _{D3} | Melanoma |
| | HJM1 | Melanoma |
| | | Medulloblastoma |
| | | Glioma |
| | | Leukemia |
| 15 | | Meninglioma |
| | 9-O-Acetyl-G _{D3} | Melanoma |
| | Fuc-G _{M1} | Small cell lung carcinoma |
| | COTA | Colon, ovarian |
| 20 | SW1038 CTS | Colon prostate |
| | MAGE-1 MAGE-2 MAGE-3 (MZ2-E MZ2- Bb) | Lung melanocyte breast |
| | MUC-1 | Breast pancreas |
| 25 | Lewis-Ag (GICA) | Ovarian myelin |
| | TAG-12 | Breast ovarian |
| | TAG-72 | colon ovarian pancrease |
| 30 | Orfan-specific cancer neoantigen (OSN) | Lung |
| | GP100 | Melanocyte |

| Antigen | Tissue |
|--|-----------------------------|
| MART-1 | Melanocyte |
| p95/p97 | Melanocyte |
| EGF receptor | Squamous tumors |
| CA125 | Ovary |
| | Breast |
| p97 (melanotransferrin) | Melanocyte |
| 22-1-1 | uterus cervix ovary |
| GA733 | gastrointestinal carcinoma |
| YH206 | adenocarcinomas |
| MART-2 | melanocytes |
| BAGE-1 | melanocytes |
| GAGE1-6 | melaocyte |
| | osteocarcoma |
| DF3 | Breast |
| | lymphocytes |
| L3p40-50 L3p90 | Lung |
| Thomsen-Friedenrich Pan Tumor Antigen | pancarcinoma |
| | pancreas |
| | ovarian |
| EPB-2 | B cell lymphoma |
| | melanoma |
| | lymphoma |
| | medullary thyroid carcinoma |
| | gastrointestinal carcinoma |

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| Antigen | Tissue |
|----------|--|
| NS-ESO-1 | melanoma, breast, bladder, prostate, heptocellular carcinoma |
| NY-ESO-1 | melanoma, breast, bladder, prostate, heptocellular carcinoma |

Proteins also include, but are not limited to, therapeutic proteins, 5 such as an immunostimulating protein, such as an interleukin, interferon, or colony stimulating factor, such as granulocyte macrophage colony stimulating factor (GM-CSF; see, e.g., 5,908,763F. Generally, such GM-CSF is a primate GM-CSF, including human GM-CSF. Other immunostimulatory genes include, but are not limited to, genes that encode 10 cytokines IL-1, IL-2, IL-4, IL-5, IFN, TNF, IL-12, IL-18, and flt3, proteins that stimulate interactions with immune cells (B7, CD28, MHC class I, MHC class II, TAPs), tumor-associated antigens (immunogenic sequences from MART-1, gp100 (pmel-17), tyrosinase, tyrosinase-related protein 1, tyrosinase-related protein 2, melanocyte-stimulating hormone receptor, 15 MAGE1, MAGE2, MAGE3, MAGE12, BAGE, GAGE, NY-ESO-1, -catenin, MUM-1, CDK-4, caspase 8, KIA 0205, HLA-A2R1701, -fetoprotein, telomerase catalytic protein, G-250, MUC-1, carcinoembryonic protein, p53, Her2/neu, triosephosphate isomerase, CDC-27, LDLR-FUT, telomerase reverse transcriptase, and PSMA), cDNAs of antibodies that 20 block inhibitory signals (CTLA4 blockade), chemokines (MIP1, MIP3, CCR7 ligand, and calreticulin), and other proteins.

Other polynucleotides, including therapeutic nucleic acids, such as therapeutic genes, of interest include, but are not limited to, anti-angiogenic, and suicide genes. Anti-angiogenic genes include, but are not limited to, genes that encode METH-1, METH -2, TrpRS fragments, proliferin-related protein, prolactin fragment, PEDF, vasostatin, various fragments of extracellular matrix proteins and growth factor/cytokine

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inhibitors. Various fragments of extracellular matrix proteins include, but are not limited to, angiostatin, endostatin, kininostatin, fibrinogen-E fragment, thrombospondin, tumstatin, canstatin, and restin. Growth factor/cytokine inhibitors include, but are not limited to, VEGF/VEGFR antagonist, sFlt-1, sFlk, sNRP1, angiopoietin/tie antagonist, sTie-2, chemokines (IP-10, PF-4, Gro-beta, IFN-gamma (Mig), IFN, FGF/FGFR antagonist (sFGFR), Ephrin/Eph antagonist (sEphB4 and sephrinB2), PDGF, TGF and IGF-1.

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A "suicide gene" encodes a protein that can lead to cell death, as with expression of diphtheria toxin A, or the expression of the protein can render cells selectively sensitive to certain drugs, e.g., expression of the Herpes simplex thymidine kinase gene (HSV-TK) renders cells sensitive to antiviral compounds, such as acyclovir, gancyclovir and FIAU $(1-(2-deoxy-2-fluoro-\beta-D-arabinofuranosil)-5-iodouracil)$. Other suicide genes include, but are not limited to, genes that encode carboxypeptidase G2 (CPG2), carboxylesterase (CA), cytosine deaminase (CD), cytochrome P450 (cyt-450), deoxycytidine kinase (dCK), nitroreductase (NR), purine nucleoside phosphorylase (PNP), thymidine phosphorylase (TP), varicella zoster virus thymidine kinase (VZV-TK), and xanthine-guanine phosphoribosyl transferase (XGPRT). Alternatively, a therapeutic nucleic acid can exert its effect at the level of RNA, for instance, by encoding an antisense message or ribozyme, a protein that affects splicing or 3' processing (e.g., polyadenylation), or a protein that affects the level of expression of another gene within the cell, e.g. by mediating an altered rate of mRNA accumulation, an alteration of mRNA transport, and/or a change in post-transcriptional regulation. The addition of a therapeutic nucleic acid to a virus results in a virus with an additional antitumor mechanism of action. Thus, a single entity (i.e., the virus carrying a therapeutic transgene) is capable of inducing multiple antitumor

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mechanisms. Other encoded proteins, include, but are not limited to, herpes simplex virus thymidine kinase (HSV-TK), which is useful as a safety switch (see, U.S. Patent Application No. 08/974,391, filed November 19, 1997, which published as PCT Publication No. WO/9925860), Nos, FasL, and sFasR (soluble Fas receptor).

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Also contemplated are combinations of two or more transgenes with synergistic, complementary and/or nonoverlapping toxicities and methods of action. The resulting adenovirus can retain the viral oncolytic functions and, for example, additionally are endowed with the ability to induce immune and anti-angiogenic responses and other responses as desired.

Therapeutic polynucleotides and heterologous polynucleotides also include those that exert an effect at the level of RNA or protein. These include a factor capable of initiating apoptosis, RNA, such as RNAi and other double-stranded RNA, antisense and ribozymes, which among other capabilities can be directed to mRNAs encoding proteins essential for proliferation, such as structural proteins, transcription factors, polymerases, genes encoding cytotoxic proteins, genes that encode an engineered cytoplasmic variant of a nuclease (e.g. RNase A) or protease (e.g. trypsin, papain, proteinase K and carboxypeptidase). Other polynucleotides include a cell or tissue specific promoters, such as those used in oncolytic adenoviruses (see, e.g., U.S. Patent No. 5,998,205).

The heterologous polynucleotide encoding a polypeptide also can contain a promoter operably linked to the coding region. Generally the promoter is a regulated promoter and transcription factor expression system, such as the published tetracycline-regulated systems, or other regulatable systems (WO 01/30843), to allow regulated expression of the encoded polypeptide. Exemplary of other promoters, are tissue-selective promoters, such as those described in U.S. Patent No. 5,998,205. An

exemplary regulatable promoter system is the Tet-On (and Tet-Off) system currently available from Clontech (Palo Alto, CA). This promoter system allows the regulated expression of the transgene controlled by tetracycline or tetracycline derivatives, such as doxycycline. This system can be used to control the expression of the encoded polypeptide in the viral particles and nucleic acids provided herein. Other regulatable promoter systems are known (see, e.g., published U.S. Application No. 20020168714, entitled "Regulation of Gene Expression Using Single-Chain, Monomeric, Ligand Dependent Polypeptide Switches," which describes gene switches that contain ligand binding domains and 10 transcriptional regulating domains, such as those from hormone receptors). Other suitable promoters that can be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter and/or the E3 promoter; or heterologous promoters, such as the 15 cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; and the ApoAl promoter.

Therapeutic transgenes can be included in the viral constructs and resulting particles. Among these are those that result in an "armed" virus. For example, rather than delete E3 region as in some embodiments described herein, all or a part of the E3 region can be preserved or re-inserted in an oncolytic adenoviral vector (discussed above). The presence of all or a part of the E3 region can decrease the immunogenicity of the adenoviral vector. It also increases cytopathic effect in tumor cells and decreases toxicity to normal cells. Typically such vector expresses more than half of the E3 proteins.

Adenoviruses for therapy, including those for human therapy, are known. Such known viruses can be modified as provided herein to

increase infection of dendritic cells and/or increasing binding to receptors expressed on dendritic cells. The adenoviral vectors that are used to produce the viral particles can include other modifications. Modifications include modifications to the adenovirus genome that is packaged in the particle in order to make an adenoviral vector. As discussed above, adenovirus vectors and particles with a variety of modifications are available. Modifications to adenvoiral vectors include deletions known in the art, such as deletions in one or more of the El, E2a, E2b, E3, or E4 coding regions. These adenoviruses are sometimes referred to as early generation adenoviruses and include those with deletions of all of the coding regions of the adenoviral genome ("gutless" adenoviruses, discussed above) and also include replication-conditional adenoviruses, which are viruses that replicate in certain types of cells or tissues but not in other types as a result of placing adenoviral genes essential for replication under control of a heterologous promoter (discussed above; see also U.S. Patent No. 5,998,205, U.S. Patent No. 5,801,029; U.S. patent application 60/348,670 and corresponding published International PCT application No. WO 02/06786). These include the cytolytic, cytopathic viruses (or vectors), including the oncolytic viruses discussed above.

Alternatively, as discussed above, the vector can include a mutation or deletion in the E1b gene. Typically such mutation or deletion in the E1b gene is such that the E1b-19kD protein becomes non-functional. This modification of the E1b region can be combined with vectors where all or a part of the E3 region is present.

H. Formulation and administration

1. Formulation

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Compositions containing therapeutically effective for concentrations of recombinant adenovirus delivery vectors for delivery of therapeutic

gene products to target cells and/or tissues (i.e. dendritic cells). Modes of administration include, but are not limited to, intramuscular, parenteral, local, topical and other routes whereby dendritic cells can be targeted.

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The recombinant viral compositions also can be formulated for in sustained released formulations, such as adsorbed to biodegradable supports, including collagen sponges, or in liposomes. Sustained release formulations can be formulated for multiple dosage administration, so that during a selected period of time, such as a month or up to about a year, several dosages are administered. Thus, for example, liposomes can be prepared such that a total of about two to up to about five or more times the single dosage is administered in one injection. The vectors are formulated in pharmaceutically acceptable carriers.

The composition can be provided in a sealed sterile vial containing an amount such that upon administration a sufficient amount of viral particles is delivered where about 50 to 150 μ l, containing at least about 10^7 , or 10^8 plaque forming units (pfu) in such volume are delivered and at least 10^9 - 10^{10} pfu are delivered.

To prepare compositions the viral particles are dialzyed into a suitable carrier or viral particles can be concentration and/or mixed therewith. The resulting mixture can be a solution, suspension or emulsion. In addition, the viral particles may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active agents for the particular disorder treated.

Exemplary suitable carriers include, but are not limited to, physiological saline, phosphate buffered saline (PBS), balanced salt solution (BSS), lactate Ringers solution, and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof. Liposomal suspensions also can be suitable as pharmaceutically acceptable carriers. These can

be prepared according to methods known to those skilled in the art.

The compositions can be prepared with carriers that protect them against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and other types of implants that may be placed directly into the body. The compositions also can be administered in pellets, such as Elvax pellets (ethylene-vinyl acetate copolymer resin).

Liposomal suspensions, including tissue-targeted liposomes, also can be suitable as pharmaceutically acceptable carriers. For example, liposome formulations may be prepared by methods known to those of skill in the art (see, e.g., Kimm et al. (1983) Bioch. Bioph. Acta 728:339-398; Assil et al. (1987) Arch Ophthalmol. 105:400; and U.S. Patent No. 4,522,811). The viral particles can be encapsulated into the aqueous phase of liposome systems.

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The active materials also can be mixed with other active materials, that do not impair the desired action, or with materials that supplement the desired action or have other action, including viscoelastic materials, such as hyaluronic acid, which is sold under the trademark HEALON, which is a solution of a high molecular weight (MW) of about 3 millions fraction of sodium hyaluronate (manufactured by Pharmacia, Inc; see, e.g., U.S. Patent Nos. 5,292,362, 5,282,851, 5,273,056, 5,229,127, 4,517,295 and 4,328,803). Additional active agents may be included.

The compositions can be enclosed in ampules, disposable syringes or multiple or single dose vials made of glass, plastic or other suitable material. Such enclosed compositions can be provided in kits. In particular, kits containing vials, ampules or other container.

Finally, the vectors can be packaged as articles of manufacture containing packaging material, typically a vial, a pharmaceutically acceptable composition containing the viral particles and a label that indicates the therapeutic use of the composition.

Also provided are kits for practice of the methods herein. The kits contain one or more containers, such as sealed vials, with sufficient composition for single dosage administration, other reagents as needed, and optionally instructions for use.

Administration of the composition is typically by intravenous or intramuscular injection, although other modes of administration can be effective.

2. Administration

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The compositions containing the compounds are generally administered systemically. It is further understood that, for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the recombinant viruses, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the methods, uses, and products provided herein.

In addition to *in vivo* administration, the viral particles provided herein can be used in methods of *ex vivo* therapy in which mixtures of cells, such as bone marrow cells, that include or contain dendritic cells or that are enriched for dendritic cells are contacted with the viral particles so that dendritic cells are preferentially infected. The resulting cells are optionally culture *in vitro* and are then infused into a recipient subject, generally the donor.

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I. Diseases, Disorders and therapeutic products

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Dendritic cells modified with adenovirual particles provided herein express heterologous proteins that can be presented or that can alter dendritic cell functioning. As noted, the adenoviral particles can be administered to a subject or can be contacted *ex vivo* with dendritic cells obtained from a donor and can be infused into a subject patient, typically the donor. The viral particles, which express fibers targeted to dendritic cells will preferentially infect dendritic cells. Dendritic cells modified to express particular antigens act as vaccines by stimulating an immune response against the presented antigen. These cells can be used for treatment or prophylaxis of virtually any bacterial, protozoan, parasitic, fungal or other infection. In addition, presentation of a tumor antigen renders such cells effective for treatment or prophylaxis of cancers.

Expression of a product that interferes with dendritic cell function, such as by blocking expression of genes, including genes encoding NFκB or RelB, prevent the dendritic cells from stimulating T-cells. Such particles and the resulting cells can be used to treat diseases such as asmtha, allergies, autoimmune diseases, such as juvenille diabetes, rheumatoid arthritis, lupus and inflammatory diseases.

Pathogens include, but are not limited to, bacterial, such as *E. coli* and anthrax, viruses, such as vaccinia virus (*i.e.* small pox, chicken pox), herpes viruses, cytomegalovirus (CMV) vectors, papillomavirus, parasites and fungi. Selected antigens can be determined empirically by identifying those that are effective in generatin an immunoprotective response in a model system such as a rodent model.

Treatment with the particles, either *in vivo* or *ex vivo* can be prophylactic where administration (vaccination) generates immunity or it can be for treatment of the disease.

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J. Examples

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

5 Construction of Ad5 Vectors Containing the Fiber AB Loop, KO1 and Penton, PD1 Mutations and Derivatives Thereof

Three recombinant adenoviral vectors were prepared that contain the KO1 fiber or PD1 penton base mutations either alone or in combination, these vectors are designated Av3nBgFKO1 Av1nBgPD1, and Av1nBgFKO1PD1. Construction of these vectors is described below and a general description of each vector is set forth in Table 1.

TABLE 1
Description Of Detargeted
Recombinant Adenoviral Vectors Used For Scale-up
Vector

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| Vector | Description |
|---------------|--|
| Av3nBg | An E1, E2a, E3-deleted adenoviral vector encoding a nuclear localizing $oldsymbol{eta}$ -galactosidase |
| Av1nBg | An E1 and E3-deleted adenoviral vector encoding a nuclear localizing $oldsymbol{eta}$ -galactosidase |
| Av3nBgFKO1 | The same as Av3nBg but containing the KO1 mutation in the fiber gene |
| Av1nBgPD1 | The same as Av1nBg but containing the PD1 mutation in the penton gene |
| Av1nBgFKO1PD1 | The same as Av1nBg but containing the fiber KO1 and penton PD1 mutations |

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Av1nBg

This is a well-known vector, its sequence is set forth in SEQ ID NO. 51.

Av3nBg

This is a well-known vector, its sequence is set forth in SEQ ID NO. 52.

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Av3nBgFKO1

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Genetic incorporation of the KO1 fiber mutation to generate Av3nBgFKO1

The adenoviral vector Av3nBgFKO1 was generated in an E1-, E2a-, E3-deleted backbone based on the adenovirus serotype 5 genome. It contains a RSV promoted nuclear-localizing β -galactosidase gene in place of the E1 region. In addition, the fiber gene carries the KO1 mutation. This mutation results in a substitution of fiber amino acids 408 and 409, changing them from serine and proline to glutamic acid and alanine, respectively.

The vector was constructed as follows. First, the plasmid pSKO1 (Figure 1) was digested with the restriction enzymes Sphl and Munl. The resulting DNA fragments were separated by electrophoresis on an agarose gel. The 1601 bp fragment containing all but the 5' end of the fiber gene was excised from the agarose gel and the DNA was isolated and purified. The fragment was then ligated with the 9236 bp fragment of p5FloxHRFRGD, which had been digested with Sphl and Munl. The resulting plasmid, p5FloxHRFKO1, was digested with Spel and Pacl and the 6867 bp fragment containing the fiber gene was isolated. The fragment was ligated with the 24,630 bp Spel-Pacl fragment of pNDSQ3.1. The resulting plasmid, pNDSQ3.1KO1 (Figure 2), was used together with pAdmireRSVnBg (Figure 3A) to generate a plasmid which encodes the full-length adenoviral vector genome. It, however, was necessary to remove the Pacl site from pNDSQ3.1KO1 (Figure 2) prior to recombination with pAdmireRSVnBg (Figure 3A) so that the final plasmid contains a unique Pacl site adjacent to the 5' ITR. The Pacl site in pNDSQ3.1KO1 was removed by digestion with Pacl followed by blunting with T4 DNA Polymerase and religation. The resulting plasmid was called pNDSQ3.1KO1(Pac.

To generate a full-length plasmid containing the entire adenoviral genome, pAdmireRSVnBg (Figure 3A) was digested with Sall and co-transfected into competent cells of the *E. coli* strain BJ5183 along with pNDSQ3.1KO1ΔPac, which had been digested with BstBl.

Homologous recombination between the two plasmids generated a full-length plasmid encoding the entire adenoviral vector genome, which was called pFLAv3nBgFKO1.

The plasmid pFLAv3nBgKO1 was linearized with Pacl and transfected into 633 cells. In the fiber complementing 633 cell line, the resulting viral DNA containing the KO1 mutation is capable of being packaged into infectious viral particles containing a mixture of wildtype fiber and mutant fiber proteins. After five rounds of amplification in 633 cells, a cytopathic effect was observed. Three more rounds of amplification in 633 cells were performed followed by purification of the virus by standard CsCl centrifugation procedures. This viral preparation was used to infect AE1-2a cells, which do not express fiber. The resulting virus contained only the mutant fiber protein on its capsid. Virus particles were purified by standard CsCl centrifugation procedures.

Av1nBgFKO1

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The v ector Av1nBgFKO1 is made in a similar manner to Av3nBgFKO1 described above.

Av1nBgKO12

An additional fiber AB loop mutation (described by Einfeld *et al.* (2001) *J. Virology* 75:11284-11291) was incorporated into the genome of Av1nBg. This AB loop mutation is a four amino acid substitution, R512S, A515G, E516G, and K517G, and is referred to as KO12. The KO12 mutation was incorporated into the fiber gene by PCR gene overlap extension using the plasmid pSQ1 (Figure 3B) as template. The pSQ1 plasmid contains most of the Ad5 genome, extending from base pair

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3329 through the right ITR, in a pBR322 backbone. First, a segment of the Ad5 genome extending from within the E3 region into the fiber gene was amplified by PCR using the plasmid pSQ1 as a template with the following primers termed 5FF, 5'-GAA CAG GAG GTG AGC TTA GA-3' SEQ ID NO. 53), and 5FR, 5'-TCC GCC TCC ATT TAG TGA ACA GTT AGG AGA TGG AGC TGG TGT G-3' (SEQ ID NO. 54). The primer 5FR contains an 18 base 5'-extension that encodes the modified fiber AB loop amino acids from 512 through 517. A second PCR using pSQ1 as a template amplified the region immediately 3' of the AB loop substitution and extending past the Munl site located 40 base pairs 3' of the fiber gene stop codon. The two primers used for this reaction were 3FF: 5'-TCA CTA AAT GGA GGC GGA GAT GCT AAA CTC ACT TTG GTC TTA AC-3' (SEQ ID NO. 55), and 3FR: 5'-GTG GCA GGT TGA ATA CTA GG-3' (SEQ ID NO. 56). The primer 3FR contains an 18 base 5'-extension that encodes the modified fiber AB loop amino acids 512 through 517. Amplified products of the expected size were obtained and used in a second PCR with the end primers 5FF and 3FR to join the fragments together. The KO12 PCR fragment was digested with Xbal and Munl cloned directly into the fiber shuttle plasmid, pFBshuttle(EcoRI) to generate the plasmid pFBSEKO12 which contains the 8.8kB EcoRI fragment of pSQ1. The pFBSEKO12 plasmid was digested with Xbal and EcoRl and cloned into pSQ1 using a three-way ligation to generate pSQ1KO12 (Figure 3C). The KO12 cDNA was incorporated into the genome of Av1nBg, an adenovirus vector with E1 and E3 deleted encoding β -galactosidase, by homologous recombination between Clal-linearized pSQ1KO12 and pAdmireRSVnBg digested with Sall and PacI to generate Av1nBgKO12. The KO12 vector was transfected in 633 cells, scaled-up on non-fiber expressing cells and purified, as described above for KO1.

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Av1nBgPD1

Genetic incorporation of the PD1 penton mutation to generate Av1nBgPD1

The adenoviral vector Av1nBgPD1 is an E1-, E3-deleted vector based on the adenovirus serotype 5 genome. It contains a RSV promoted nuclear-localizing β -galactosidase gene in the E1 region and also contains the PD1 mutation in the penton gene. The PD1 mutation results in a substitution of amino acids 337 through 344 of the penton protein, HAIRGDTF (SEQ ID NO. 49), with amino acids SRGYPYDVPDYAGTS (SEQ ID NO. 50), thus replacing the RGD tripeptide (see, Einfeld et al. 10 (2001) J. Virology 75:11284-11291). The mutation in the penton gene was generated in the plasmid pGEMpen5, which contains the Adenovirus serotype 5 penton gene. To generate the mutation, four oligonucleotides were synthesized. The sequences of the oligonucleotides were as follows: penton 1: 5' CGC GGA AGA GAA CTC CAA CGC GGC AGC 15 CGC GGC AAT GCA GCC GGT GGA GGA CAT GAA 3' (SEQ ID NO. 57); penton 2: 5' TAT CGT TCA TGT CCT CCA CCG GCT GCA TTG CCG CGG CTG CCG CGT TGG AGT TCT CTT CC 3' (SEQ ID NO. 58); penton 3: 5' CGA TAG CCG CGG CTA CCC CTA CGA CGT GCC CGA CTA CGC GGG CAC CAG CGC CAC ACG GGC TGA GGA GAA GCG CGC 3' (SEQ 20 ID NO. 59); penton 4: 5' TCA GCG CGC TTC TCC TCA GCC CGT GTG GCG CTG GTG CCC GCG TAG TCG GGC ACG TCG TAG GGG TAG CCG CGG C 3' (SEQ ID NO. 60). The complementary oligonucleotides penton 1 and penton 2 were annealed to each other as were penton 3 and penton 4. The duplex generated by annealing penton 3 and penton 4 25 encoded the substitution of amino acids 337 through 344 described above. The duplex generated by annealing penton 1 and penton 2 possessed a 5 base 5' overhang which was compatible to a 5 base 5' overhang on the duplex generated by annealing penton 3 and penton 4.

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The opposite end of the duplex generated by annealing penton 1 and penton 2 contained an Earl compatible overhang. The opposite end of the duplex generated by annealing penton 3 and penton 4 contained a BbvCl compatible overhang. The two duplexes were ligated to each other and ligated back into the pGEMpen5 backbone as follows. First, pGEMpen5 was digested with BbvCl and Pstl and the resulting DNA fragments were separated by electrophoresis on an agarose gel. The 3360 bp fragment was excised from the gel and purified. The plasmid pGEMpen5 was also digested with Pstl and Earl and the resulting fragments were separated by electrophoresis on an agarose gel. The 955 bp fragment was excised from the gel and purified. These two fragments from the pGEMpen5 plasmid were ligated with the two pairs of annealed oligonucleotides to generate the plasmid pGEMpen5PD1.

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The mutated penton gene was transferred from pGEMpen5PD1 to pSQ1 using a 5-way ligation as follows. First, the region of the penton gene containing the PD1 mutation was excised from pGEMpen5PD1 by digestion with Pvul and Ascl. The 974 bp fragment containing the PD1 mutation was purified. Four DNA fragments were prepared from the pSQ1 plasmid (Figure 3B) as follows. The plasmid was digested with Csp45I and Fsel and the 9465 bp fragment was purified. In addition pSQ1 was digested with Fsel and Pvul and the 2126 bp fragment was purified. The plasmid pSQ1 was digested with Ascl and BamHI and the 5891 bp fragment was purified. Finally, pSQ1 was digested with BamHI and Csp45I and the 14610 bp fragment was purified. The 5 purified DNA fragments were ligated to each other to form the plasmid pSQ1PD1 (Figure 4).

To generate adenoviral vector, pSQ1PD1 was linearized by digestion with Clal and co-transfected into PerC6 cells with pAdmireRSVnBg (Figure 3A) which had been digested with Sall and Pacl.

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hexadimethrine bromide was maintained in the medium at 4 μ g/ml. When a cytopathic effect was observed, a crude viral lysate was further expanded on PerC6 cells. The virus was purified by standard CsCl centrifugation procedures.

5 Av1nBgFKO1PD1

Genetic incorporation of the fiber KO1 or KO12 mutation in combination with the penton PD1 mutation to generate Av1nBgFKO1PD1

The adenoviral vectors Av1nBgFKO1PD1 and Av1nBgKO12PD1 10 were generated in an E1-, E3-deleted adenovirus serotype 5 genome. Both vectors contains a RSV promoted nuclear-localizing β -galactosidase gene in the E1 region and also contains either the KO1 or KO12 mutation in the fiber gene as well as the PD1 mutation in the penton gene. The vectors were constructed as follows. First, the plasmid pSQ1PD1 was 15 digested with Csp45I and Spel and the 23976 bp fragment containing the PD1 mutated penton gene was purified. In addition, the plasmids pSQ1KO1 or pSQ1KO12 (Figure 3B) were digested with Csp45I and SpeI and the 9090 bp fragment containing the KO1 or KO12 mutated fiber gene were purified. The appropriate purified fragments were ligated to 20 each other to from the plasmid pSQ1FKO1PD1 (Figure 5A) or pSQ1KO12PD1 (Figure 5B) that contains the KO1 (or KO12) mutated fiber gene and the PD1 mutated penton gene. To generate virus, pSQ1FKO1PD1 or pSQKO12PD1 was linearized with Clal and co-transfected into 633 cells with pAdmireRSVnBg (Figure 3A) which had been 25 digested with Sall and Pacl. After three rounds of amplification in 633 cells a cytopathic effect was observed and the crude viral lysate was then amplified on PerC6 cells. Hexadimethrine bromide was maintained in the medium at 4 μ g/ml. Each virus was purified by standard CsCl centrifugation procedures.

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EXAMPLE 2

In Vitro Evaluation of Adenoviral Vectors Containing the KO1 and PD1 Mutations

Several recombinant adenoviral vectors were used in these studies to demonstrate the function of the KO1 fiber mutation and included Av1nBg, Av1nBgFKO1, Av1nBgPD1, and Av1nBgFKO1PD1, described above. The transduction efficiencies of adenoviral vectors containing the KO1 and/or PD1 mutations were evaluated on cells of the alveolar epithelial cell line A549. The transduction efficiencies were compared to that of Av1nBg, an adenoviral vector containing wild type fiber and penton.

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The day prior to infection, cells were seeded into 24-well plates at a density of approximately 1 x 10^5 cells per well. Immediately prior to infection, the exact number of cells per well was determined by counting a representative well of cells. Each of the vectors, Av1nBg, Av1nBgFKO1, and Av1nBgFKO1PD1 were used to transduce A549 cells at each of the following particle per cell (PPC) ratios: 100, 500, 1000, 2500, 5000, 10,000. The cell monolayers were stained with X-gal 24 hours after infection and the percentage of cells expressing β -galactosidase was determined by microscopic observation and counting of cells. Transductions were done in triplicate and three random fields in each well were counted, for a total of nine fields per vector.

The results at the 500 PPC ratio are shown in Figure 6 and show a significantly reduced transduction efficiency on A549 cells using vectors containing the KO1 mutation alone or when combined with PD1 compared to Av1nBg. The vectors containing the PD1 mutation alone had no effect on adenoviral transduction of A549 cells *in vitro*.

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EXAMPLE 3

In Vivo Analysis of Adenoviral Vectors Containing the FKO1 and PD1 Mutations

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This Example provides experiments that evaluate the *in vivo* biodistribution of adenoviral vectors containing the KO1 and PD1 mutations and their influence on adenoviral-mediated liver transduction. The results show that ablating the viral interaction with CAR and/or integrins is not sufficient to fully detarget adenoviral vectors from the liver *in vivo*.

A positive control cohort received Av1nBg and a negative control group received HBSS. Additionally, the Av1nBgFKO12 and Av1nBgFKO12PD1 vectors were analyzed in vivo. These vectors each contain a fiber protein with the four amino acid substitution in the AB loop. Additionally, Av1nBgFKO12PD1 contains a mutation in the penton base. Both of these mutations were known (see, Einfeld et al. (2001) J. Virology 75:11284-11291), and were alleged to decrease liver transduction 10 to 700 fold, respectively. Cohorts of five C57BL/6 mice received each vector via tail vein injection at a dose of 1 x 1013 particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. The median lobe of the liver was placed in neutral buffered formalin to preserve the sample for β-galactosidase immunohistochemistry. In addition, tissue from each organ was frozen to preserve it for hexon PCR analysis to determine vector content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay.

For β -galactosidase immunohistochemistry slices of liver, approximately 2-3 mm thick, were placed in 10% neutral buffered formalin. After fixation, these samples were embedded in paraffin,

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sectioned, and analyzed by immunohistochemistry for β-galactosidase expression. A 1:1200 dilution was used of a rabbit anti-β-galactosidase antibody (ICN Pharmaceuticals, Inc.; Costa Mesa, CA) in conjunction with a Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA) to visualize positive cells.

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The chemiluminescent β -galactosidase activity assay was performed using the Galacto-Light PlusTM chemiluminescent assay (Tropix, Inc., Foster City, CA) system. Tissue samples were collected in lysis matrix tubes containing two ceramic spheres (Bio101, Carlsbad, CA) and frozen on dry ice. The tissues were thawed and 500 μ l of lysis buffer from the Galacto-Light Plus kit was added to each tube. The tissue was homogenized for 30 seconds using a FastPrep System (Bio101, Carlsbad, CA). Liver samples were homogenized for an additional 30 seconds. β -galactosidase activity was determined in the liver homogenates according to the manufacture's protocol.

For hexon PCR analysis DNA from tissues was isolated using the Qiagen Blood and Cell Culture DNA Midi or Mini Kits (Qiagen Inc., Chatsworth, CA). Frozen tissues were partially thawed and minced using sterile disposable scalpels. Tissues were then lysed by incubation overnight at 55° C in Qiagen buffer G2 containing 0.2 mg/ml RNaseA and 0.1 mg/ml protease. Lysates were vortexed briefly and then applied to Qiagen-tip 100 or Qiagen-tip 25 columns. Columns were washed and DNAs were eluted as described in the manufacturer's instructions. After precipitation, DNAs were dissolved in water and the concentrations were spectrophotometrically determined (A260 and A280) on a DU-600 (Beckman Coulter, Inc.; Fullerton, CA) or a SPECTRAmax PLUS (Molecular Devices, Inc.; Sunnyvale, CA) spectrophotometer. 2.3.2.

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PCR primers and a Taqman probe specific to adenovirus hexon sequences were designed using Primer Express software v. 1.0 (Applied Biosystems, Foster City, CA). Primer and probe sequences were:

Hexon Forward Primer (SEQ ID NO. 61):

5'-CTTCGATGATGCCGCAGTG-3'

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Hexon Reverse Primer (SEQ ID NO. 62):

5'-GGGCTCAGGTACTCCGAGG-3'

Hexon Probe (SEQ ID NO. 63):

5'-FAM-TTACATGCACATCTCGGGCCAGGAC-TAMRA-3'

Amplification was performed in a reaction volume of 50 μ l under the following conditions: 10 ng (tumor) or 1 μ g (liver and lung) of sample DNA, 1X Tagman Universal PCR Master Mix (Applied Biosystems), 600 nM forward primer, 900 nM reverse primer and 100 nM hexon probe. Thermal cycling conditions were: 2 minute incubation at 50° C, 10 minutes at 95° C, followed by 35 cycles of successive incubation at 95° C for 15 seconds and 60° C for 1 minute. Data was collected and analyzed using the 7700 Sequence Detection System software v. 1.6.3 (Applied Biosystems). Quantification of adenovirus copy number was performed using a standard curve that includes dilutions of adenovirus DNA from 1,500,000 copies to 15 copies in the appropriate background 20 of cellular genomic DNA. For analysis of tumor tissues, a standard curve in a background of 10 ng human DNA was generated. For analysis of mouse liver and lung tissues, a standard curve using the same adenovirus DNA dilutions in a background of 1 μ g CD-1 mouse genomic DNA was generated. Samples were amplified in triplicate, and the average number of total copies was normalized to copies per cell based on the input DNA weight amount and a genome size of 6 x 109 bp.

The results of the β -galactosidase activity assay and adenoviral hexon DNA content for liver transduction by these vectors are shown in

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Figure 7A and 7B. The vector containing the KO1 or KO12 mutations alone showed, on average, a slight increase in liver transduction compared to Av1nBg, which is consistent with several previous experiments. The vectors containing the PD1 mutation alone or combined with KO1 or KO12 showed a slight decrease in liver transduction compared to Av1nBg, suggesting that integrins are involved to some extent in hepatic uptake of the adenoviral vectors.

The results of the immunohistochemical staining of liver sections for β-galactosidase were consistent with the activity assays (data not shown) and demonstrate that gene expression was localized specifically to hepatocytes. The vectors containing the KO1 or KO12 mutation alone showed a slight increase in liver transduction as revealed by a more intense and frequent immunohistochemical-staining pattern. The vectors containing the PD1 mutation, either alone or combined with KO1 or KO12, showed little difference in transduction compared to Av1nBg. These results demonstrate that ablating the viral interaction with CAR and/or integrins is not sufficient to fully detarget adenoviral vectors from the liver *in vivo*.

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In summary, the fiber AB loop mutation contained in Av1nBgFKO1 or Av1nBgKO12 ablates interaction with human and mouse CAR *in vitro* and diminished transduction *in vitro*. *In vivo*, however, fiber AB loop mutations behaved unexpectantly, because such mutations were found to enhance adenoviral-mediated gene transfer to liver and results in increasing vector potency. The penton base, PD1 mutation that ablates interaction with the second receptor involved in adenoviral internalization had no effect *in vitro* and little to no effect *in vivo*. These studies indicated that other receptors are responsible for adenoviral gene transfer to the liver *in vivo*.

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EXAMPLE 4

Description Of Adenoviral Vectors Containing A Fiber With Amino Acid Substitutions At The Heparin Sulfate Binding Domain In The Fiber Shaft

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Vectors containing substitutions at all four of the amino acids in the four amino acid motif in the Ad5 fiber shaft (residues 91 to 94, KKTK; SEQ ID NO. 45) were generated in order to ablate the potential interaction with HSP. The mutation is termed HSP because it potentially eliminates binding to heparan sulfate proteoglycans. Vectors containing the HSP mutation alone and combined with the KO1 mutation (fiber knob AB loop mutation that ablates CAR binding), the PD1 mutation (penton mutation that eliminates RGD/integrin interaction), and a triple knockout vector (HSP, KO1, PD1) were generated.

Generation of the HSP fiber mutation: The HSP mutation was

incorporated into the fiber gene by using a PCR-based strategy of gene splicing by overlap extension (PCR SOEing). First, a segment of the Ad5 15 genome extending from within the E3 region into the 5' end of the fiber gene was amplified by PCR using the plasmid pSQ1 (Figure 3B) as a template and two primers termed 5FF and 5HSPR. The DNA sequence of 5FF is as follows: 5' GAA CAG GAG GTG AGC TTA GA 3' (SEQ ID NO. 20 53). This sequence corresponds to base pairs 25,199 - 25,218 of pSQ1. The DNA sequence of 5HSPR is as follows: 5' GGC TCC GGC TCC GAG AGG TGG GCT CAC AGT GGT TAC ATT T 3' (SEQ ID NO. 64). 5HSPR is a reverse primer for 5FF and corresponds to a region in the fiber shaft adjacent to the KKTK (SEQ ID NO. 45) region. The primer contains a 5' 25 extension that encodes a GAGA substitution for the native KKTK (encoded by SEQ ID NO. 45) amino acid sequence. A second PCR using pSQ1 as a template amplified the region immediately 3' of the KKTK (SEQ ID NO. 45) site and extending past the Munl site located 40 base pairs 3' of the stop codon for the fiber gene. The two primers used for this

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reaction were 3HSPF and 3FR. The DNA sequence of 3HSPF is as follows: 5' GGA GCC GGA GCC TCA AAC ATA AAC CTG GAA AT 3' (SEQ ID NO. 16). It contains a 5' extension that is complementary to the 5' extension of 5HSPR. The DNA sequence of 3FR is as follows: 5' GTG GCA GGT TGA ATA CTA GG 3' (SEQ ID NO. 56).

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The two PCR products were joined by PCR SOEing using primers 5FF and 3FR. The resulting PCR product was digested with the restriction enzymes Xbal and Munl. The 2355 bp fragment was gel purified and ligated with the 6477 bp Xbal to Munl fragment of the plasmid pFBshuttle(EcoRl) (Figure 8) to generate the plasmid pFBSEHSP. The plasmid pFBshuttle(EcoRl) was generated by digesting the plasmid pSQ1 with EcoRl, then gel purifying and self-ligating the 8.8 kb fragment containing the fiber gene. Next, the fiber gene containing the HSP mutation was transferred from pFBSEHSP into pSQ1 using a three-way ligation. The 16,431 bp EcoRl to Ndel fragment of pSQ1, the 9043 bp Ndel to Xbal fragment of pSQ1, and the 7571 bp Xbal to EcoRl fragment of pFBSEHSP were isolated and ligated to generate pSQ1HSP (Figure 9).

To generate a recombinant adenoviral vector containing the HSP mutation in the fiber gene, pSQ1HSP was digested with Clal and pAdmireRSVnBg (Figure 3A) was digested with Sall and Pacl, then the two digested plasmids were co-transfected into 633 cells (von Seggern et al. (2000) J Virology 74:354-362). Homologous recombination between the two plasmids generated a full-length adenoviral genome capable of replication in 633 cells, which inducibly express Ad5 E1A and constitutively express wild-type fiber protein. After propagation on 633 cells, the virus capsid contained wildtype and mutant fiber proteins. To obtain viral particles containing only the modified fiber with the HSP mutation, the viral preparation was used to infect PerC6 cells, which do

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not express fiber. The resulting virus, termed Av1nBgFS*, was purified by standard CsCl centrifugation procedures.

Generation of vector containing the HSP and KO1 mutations

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To generate an adenoviral vector containing the HSP and KO1 mutations in fiber, a PCR SOEing strategy identical to the one described above was used except that the plasmid pSQ1FKO1 was used as the template. The PCR SOEing product was digested with Xbal and Munl and ligated with the 6477 bp Xbal to Munl fragment of pFBshuttle(EcoRl) to generate pFBSEHSPKO1. The fiber gene containing the HSP and KO1 mutations was transferred from pFBSEHSPKO1 into the pSQ1 backbone using a three-way ligation strategy identical to the one described above for the HSP mutation alone, to generate the plasmid pSQ1HSPKO1 (Figure 10). Recombinant adenoviral vector containing the HSP and KO1 mutations in the fiber gene was generated by co-transfecting pSQ1HSPKO1 digested with Clal and pAdmireRSVnBg digested with Sall and Pacl into 633 cells. Adenovirus was propagated and purified as described above for the vector containing the HSP mutation alone. The resulting virus was termed Av1nBgFKO1S*.

Generation of vector containing the HSP and PD1 mutations

The following strategy was used to generate a recombinant adenoviral vector containing the fiber HSP mutation and the penton PD1 mutation. The plasmid pSQ1PD1 (Figure 4) was digested with the restriction enzymes Csp45I and SpeI and the 23,976 bp fragment was isolated and purified. In addition, the plasmid pSQ1HSP was also digested with Csp45I and SpeI and the 9090 bp fragment was isolated and purified and ligated to the 23,976 bp fragment to generate the plasmid pSQ1HSPPD1 (Figure 11), which contains the fiber HSP and penton PD1 mutations. An adenoviral vector was generated, propagated,

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and purified as described above. The resulting virus was termed Av1nBgS*PD1.

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Generation of vector containing the HSP, KO1, and PD1 mutations

To generate an adenoviral vector containing the HSP, KO1, and PD1 mutations the following strategy was used. First, the plasmid pSQ1PD1 was digested with Csp45I and Spel and the 23,976 bp fragment was isolated and purified. In addition, the plasmid pSQ1HSPKO1 was digested with Csp45I and Spel and the 9090 bp fragment was isolated and purified. The two DNA fragments were ligated to form the plasmid pSQ1HSPKO1PD1 (Figure 12). Recombinant adenoviral vector was generated, propagated, and purified as described above. The resulting virus was termed Av1nBgFKO1S*PD1.

EXAMPLE 5

In Vitro Evaluation Of Adenoviral Vectors Containing The HSP Fiber15 Mutation

The transduction efficiencies of adenoviral vectors containing the HSP mutation in the fiber gene, either alone or combined with the KO1 and/or PD1 mutations, were evaluated on A549 and HeLa cells. The transduction efficiencies were compared to that of Av1nBg, an adenoviral vector containing wild type fiber and penton. The day prior to infection, cells were seeded into 24-well plates at a density of approximately 1 x 10⁵ cells per well. Immediately prior to infection, the exact number of cells per well was determined by counting a representative well of cells. Each of the vectors, Av1nBg (see, Stevenson *et al.* (1997) *J. Virol.* 71:4782-4790), Av1nBgS*, Av1nBgFKO1S*, Av1nBgS*PD1, and Av1nBgFKO1S*PD1, were used to transduce A549 cells at each of the following particle per cell (PPC) ratios: 100, 500, 1000, 2500, 5000, 10,000. HeLa cells were transduced with each of the above vectors, as well as a vector containing the KO1 mutation alone (Av1nBgFKO1) and a

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vector containing the PD1 mutation alone (Av1nBgPD1) at 2000 PPC. The cell monolayers were stained with X-gal 24 hours after infection and the percentage of cells expressing β -galactosidase was determined by microscopic observation and counting of cells. Transductions were done in triplicate and three random fields in each well were counted, for a total of nine fields per vector.

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The results (depicted in Figures 13A-13B) showed significantly reduced transduction efficiencies on A549 and HeLa cells using vectors containing the HSP mutation compared to Av1nBg. The vectors containing the HSP mutations, however, demonstrated a dose response on A549 cells, in that increasing PPC ratios yielded increasing transduction.

Competition experiments were done to determine which receptor molecular interactions are involved in transduction of A549 cells by the various vectors. Transductions were performed in the presence or absence of various competitors including Ad5 fiber knob, a 50 amino acid oligopeptide derived from Adenovirus serotype 2 penton base which spans the RGD tripeptide region, or heparin (Invitrogen Life Technologies, Gaithersburg, MD). Monolayers of A549 cells were cultured in Richters medium supplemented with 10% FBS and were transduced with Av1nBg, Av1nBgS*, Av1nBgFKO1S*, Av1nBgS*PD1, or Av1nBgFKO1S*PD1 in infection medium (IM, Richters medium plus 2% FBS). Different PPC ratios were used for the different vectors to achieve measurable transduction levels. The PPC ratios were as follows: Av1nBg: 500 PPC, Av1nBgS*: 10,000 PPC, Av1nBgFKO1S*: 20,000 PPC, Av1nBgS*PD1: 10,000 PPC, and Av1nBgFKO1S*PD1: 20,000 PPC. Fiber knob competition was performed by pre-incubating cells in IM containing 16 μ g/ml of fiber knob for 10 minutes at room temperature prior to infection with virus. Penton base peptide competition was performed by

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pre-incubating cells in IM containing 500nM peptide for 10 minutes at room temperature prior to infection with virus. Heparin competition was performed by pre-incubating each adenoviral vector in IM containing 3 mg/ml of heparin for 20 minutes at room temperature. In all cases, the competitor remained in the IM during the 1 hour infection when virus was rocked on the cell monolayers at 37° C in 5% CO2. After infection, the monolayers were washed with PBS, 1 ml of complete medium was added per well and the cells were incubated for an additional 24 hours to allow for β -galactosidase expression. The cell monolayers were then fixed and stained with X-Gal. The percentage of cells transduced was determined by light microscopy as described above. Each condition was carried out in triplicate and three random fields per well were counted, for a total of nine fields per condition. The average percentage of transduction per high-power field was determined.

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The results of the competition experiment (Figure 13C) showed that fiber knob inhibited transduction of cells by all vectors except for those that contained the KO1 mutation. The penton base peptide only inhibited transduction by Av1nBgFKO1S*. Heparin inhibited transduction by Av1nBgFKO1S* and Av1nBgFKO1S*PD1, but did not affect transduction by any of the other viruses suggesting the presence of additional heparin binding sites on the adenoviral capsid but that the shaft contains the predominant site.

EXAMPLE 6

In Vivo Analysis Of Adenoviral Vectors Containing The HSP Mutation In25 Fiber

The objective of this study was to evaluate the *in vivo* biodistribution of adenoviral vectors containing the HSP mutation and to determine whether this shaft modification influences adenoviral-mediated liver transduction. In addition, vectors containing the HSP mutation

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combined with KO1, or PD1, or a combination of all three mutations were evaluated as well as vectors containing the KO1 mutation alone and the PD1 mutation alone. A positive control cohort received Av1nBg and a negative control group received HBSS. Cohorts of five C57BL/6 mice received each vector via tail vein injection at a dose of 1 x 10¹³ particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. The median lobe of the liver was placed in neutral buffered formalin to preserve the sample for B-galactosidase immunohistochemistry. In addition, tissue from each organ was frozen to preserve it for hexon real time PCR analysis to determine vector content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. β -galactosidase immunohistochemistry, hexon real-time PCR and the chemiluminescent B-galactosidase activity assay were carried out as described in Example 3.

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The results of the β -galactosidase activity assay (Figure 14A) and adenoviral hexon DNA content (Figure 14B) showed a dramatic reduction in liver transduction by vectors containing the HSP mutation. The vectors containing the HSP mutation alone resulted in reducing adenoviral-mediated liver gene expression by approximately 20-fold. When combined with the KO1 mutation (HSP, KO1, PD1), yielded approximately a 1000-fold reduction in β -galactosidase activity in the liver compared to the control vector Av1nBg. The vector containing the KO1 mutation alone showed a slight increase, on average, in liver transduction compared to Av1nBg, which is consistent with several previous experiments. The vectors containing the PD1 mutation alone or combined with KO1 showed a slight decrease in liver transduction compared to Av1nBg, although the decrease was not statistically significant. Analysis

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of hepatic adenoviral hexon DNA content (Figure 14B) confirmed these results.

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The results of the immunohistochemical staining of liver sections for β -galactosidase were consistent with the activity assays (data not shown) and demonstrated that gene expression was localized specifically to hepatocytes. Vectors containing the HSP mutation, either alone or in combination with KO1 and/or PD1, showed a dramatic reduction in hepatocyte transduction. The vector containing the KO1 mutation alone showed a slight increase in liver transduction as revealed by a more intense and frequent immunohistochemical staining pattern. The vectors containing the PD1 mutation, either alone or combined with KO1, showed little difference in transduction compared to Av1nBg.

EXAMPLE 7

Description of Adenoviral Vectors Containing the HSP Fiber Shaft

Mutation with and without the KO1 Fiber Mutation and with and without a cRGD Targeting Ligand in the Fiber Knob HI Loop

Generation of vector containing the HSP fiber shaft mutation and a cRGD ligand in the HI loop: The following strategy was used to generate an adenoviral vector containing a fiber with the HSP shaft mutation and a cRGD ligand in the HI loop. The plasmid p5FloxHRFRGD was digested with the restriction enzymes BstXl and Kpnl and the 1157 bp fragment was isolated and purified. In addition, the fiber shuttle plasmid pFBSEHSP, described in Example 1 above, was digested with BstXl and Kpnl and the 4549 bp and 3156 bp fragments were isolated and purified.

The three fragments were ligated to generate the plasmid pFBSEHSPRGD, which encodes a fiber containing the HSP mutation and cRGD in the HI loop. The fiber gene from this plasmid was transferred into the pSQ1 backbone as follows. The plasmid pFBSEHSPRGD was digested with EcoRl and Xbal and the 7601 bp fragment was isolated and purified. The plasmid pSQ1 (Figure 3B) was digested with the restriction enzymes

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EcoRI, Ndel, and Xbal and the 16,431 bp EcoRI to Ndel fragment and the 9043 bp Ndel to Xbal fragment were isolated and purified. The three DNA fragments were ligated to generate the plasmid pSQ1HSPRGD (Figure 15A).

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To generate a recombinant adenoviral vector containing the HSP mutation in the fiber gene along with a cRGD ligand in the HI loop, the plasmid pSQ1HSPRGD was digested with Clal and co-transfected into 633 cells with pAdmireRSVnBg which had been digested with Sall and Pacl. After propagation on 633 cells, the virus capsid contained wildtype and mutant fiber proteins. To obtain viral particles containing only the modified fiber with the HSP mutation and a cRGD ligand, the viral preparation was used to infect PerC6 cells, which do not express fiber. The resulting virus, termed Av1nBgS*RGD, was purified by standard CsCl centrifugation procedures.

15 Generation of vector containing the HSP fiber shaft mutation, the KO1 fiber knob mutation, and a cRGD ligand in the HI loop

The following strategy was used to generate an adenoviral vector containing a fiber with the HSP shaft mutation, the KO1 fiber knob mutation, and a cRGD ligand in the HI loop. The plasmid p5FloxHRFRGD was digested with the restriction enzymes BstXI and KpnI and the 1157 bp fragment was isolated and purified. In addition, the fiber shuttle plasmid pFBSEHSPKO1, described in Example 1 above, was digested with BstXI and KpnI and the 4549 bp and 3156 bp fragments were isolated and purified. The three fragments were ligated to generate the plasmid pFBSEHSPKO1RGD, which encodes a fiber containing the HSP mutation, the KO1 mutation, and cRGD in the HI loop. The fiber gene from this plasmid was transferred into the pSQ1 backbone as follows. The plasmid pFBSEHSPKPO1RGD was digested with EcoRI and XbaI and the 7601 bp fragment was isolated and purified. The plasmid pSQ1 (Figure 3B) was

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digested with the restriction enzymes EcoRI, NdeI, and XbaI and the 16,431 bp EcoRI to NdeI fragment and the 9043 bp NdeI to XbaI fragment were isolated and purified. The three DNA fragments were ligated to generate the plasmid pSQ1HSPKO1RGD (Figure 15B).

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To generate a recombinant adenoviral vector containing the HSP and KO1 mutations in the fiber gene along with a cRGD ligand in the HI loop, the plasmid pSQ1HSPKO1RGD was digested with Clal and co-transfected into 633 cells with pAdmireRSVnBg which had been digested with Sall and Pacl. After propagation on 633 cells, the virus capsid contained wildtype and mutant fiber proteins. To obtain viral particles containing only the modified fiber with the HSP and KO1 mutations and a cRGD ligand, the viral preparation was used to infect PerC6 cells, which do not express fiber. The resulting virus, termed Av1nBgFKO1S*RGD, was purified by standard CsCl centrifugation procedures.

EXAMPLE 8

In Vitro Evaluation of Adenoviral Vectors Containing the HSP Fiber Shaft Mutation with or without the Fiber Knob KO1 Mutation and with or without a cRGD Ligand in the HI Loop

The transduction efficiencies of adenoviral vectors containing the HSP fiber shaft mutation with or without the fiber KO1 mutation and with or without the cRGD ligand in the HI loop were evaluated on A549 cells. The transduction efficiencies were compared to that of Av1nBg, an adenoviral vector containing wild type fiber. The day prior to infection, cells were seeded into 24-well plates at a density of approximately 1 x 10⁵ cells per well. Immediately prior to infection, the exact number of cells per well was determined by counting a representative well of cells. Each of the vectors, Av1nBg, Av1nBgS*, Av1nBgFKO1S*, Av1nBgFKO1S*, Av1nBgFKO1S*, and Av1nBgFKO1S*RGD, were used to transduce A549 cells at a particle to cell ratio of 6250. The cell monolayers were stained

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with X-gal 24 hours after infection and the percentage of cells expressing β-galactosidase was determined by microscopic observation and counting of cells. Transductions were done in triplicate and three random fields in each well were counted, for a total of nine fields per vector. The results (Figure 16) showed that the cRGD ligand dramatically increased the transduction efficiencies of vectors containing the HSP mutation alone or combined with the KO1 mutation. Av1nBgS* yielded approximately 22% positive cells, while Av1nBgS*RGD yielded approximately 95% positive cells. Similarly, Av1nBgFKO1S* yielded only 4% positive cells, while Av1nBgFKO1S*RGD yielded 85% positive cells. Therefore, the vector containing the shaft mutation is viable and can be retargeted with the addition of a ligand.

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EXAMPLE 9

Construction Of Ad5 Vectors Containing The Ad35 Fiber And Derivatives 15 Thereof

The KO1 and HSP mutations in the Ad5 fiber protein (5F), described above, were designed to ablate interactions that are responsible for the normal tropism of the Ad5 virus. An alternative strategy to detarget the virus is to replace the Ad5 fiber with a fiber from another serotype which does not bind CAR and which does not possess the heparin sulfate proteoglycan (HSP) binding domain (KKTK; SEQ ID NO. 45) within the shaft. The fiber of adenovirus serotype 35 (35F) does not bind CAR and does not possess the HSP binding domain in its shaft. Replacement of the 5F with the 35F can detarget the liver and provide a suitable platform for retargeting the vector to the desired tissue.

Generation of an Ad5 based vector containing the Ad35 fiber: A PCR SOEing strategy was used to generate a vector based on the Ad5 serotype but containing the Ad35 fiber in place of the Ad5 fiber. First, PCR was used to amplify a region in the plasmid pSQ1 between the Xbal

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site at bp 25,309 and the start of the fiber gene. The primers used for this reaction were P-0005/U and P-0006/L. The DNA sequence of P-0005/U was as follows: 5' C TCT AGA AAT GGA CGG AAT TAT TAC AG 3' (SEQ ID NO. 65). This sequence corresponds to bp 25,308 5 through 25,334 of pSQ1. The DNA sequence of P-0006/L was as follows: 5' TCT TGG TCA TCT GCA ACA ACA TGA AGA TAG TG 3' (SEQ ID NO. 66). It contains a 10 base pair 5' extension that is complementary to the start of the Ad35 fiber gene, while the remainder of the primer anneals to the sequence immediately 5' of the ATG start codon 10 of the fiber gene in pSQ1. A PCR product of the expected size, 583 bp, was obtained and the DNA was gel purified. A second PCR amplified the Ad35 fiber gene using DNA extracted from wildtype Ad35 virus as a template. The primers used for this reaction were P-0007/U and 35FMun. The DNA sequence of P-0007/U was as follows: 5' GT TGT 15 TGC AG ATG ACC AAG AGA GTC CGG CTC A 3' (SEQ ID NO. 67). It contains a 10 base pair 5' extension that is homologous to the 10 bp immediately prior to the ATG start codon of the fiber gene in Ad5. The remainder of the primer anneals to the start of the Ad35 fiber gene. The DNA sequence of 35FMun was as follows: 5' AG CAA TTG AAA AAT 20 AAA CAC GTT GAA ACA TAA CAC AAA CGA TTC TTT A GTT GTC GTC TTC TGT AAT GTA AGA A 3' (SEQ ID NO. 68). It contains a 46 base pair 5' extension that is complementary to the region of the Ad5 genome between the end of fiber and the Munl site 40 bp downstream of the fiber gene. In addition, the 5' extension encodes the last amino acid 25 and stop codon of the Ad5 fiber gene. This region was retained in the vector because it contains the polyadenylation site for the fiber gene. The remainder of the primer anneals to the 3' end of the Ad35 fiber gene, up to the next to last amino acid codon. A PCR product of the expected size, 1027 bp, was obtained and the DNA was gel purified. The two PCR

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products were mixed and joined together by PCR SOEing using primers P-0005/U and P-0009. The DNA sequence of P-0009 was as follows: 5' AG CAA TTG AAA AAT AAA CAC GTT G 3' (SEQ ID NO. 69). It corresponds to bp 27,648 through 27,669 of pSQ1 and overlaps the Munl site in that region. A PCR product of the expected size, 1590 bp, 5 was obtained and gel purified. It was cloned into the plasmid pCR4blunt-TOPO (Invitrogen Corporation, Carlsbad CA) using the Zero Blunt TOPO PCR Cloning Kit from Invitrogen. This intermediate cloning step simplified DNA sequencing of the PCR SOEing product. The 10 resulting plasmid, termed pTOPOAd35F, was digested with Xbal and Munl and the 1585 bp digestion product was gel purified and ligated with the 6477 bp fragment of pFBshuttle(EcoRI) digested with Xbal and MunI to generate the plasmid pFBshuttleAd35F. The Ad35 fiber gene was transferred from pFBshuttleAd35F into pSQ1 as follows. The plasmid 15 pSQ1 was digested with EcoRl and the 24,213 bp fragment was gel purified. The plasmid pFBshuttleAd35F was linearized with EcoRI and ligated with the 24,213 bp fragment from pSQ1. Restriction diagnostics were performed to screen for constructs containing the Ad35 fiber gene inserted into the pSQ1 backbone in the correct orientation. The pSQ1 20 plasmid containing the Ad35 fiber gene in the proper orientation was termed pSQ1Ad35Fiber (Figure 17A). To generate adenoviral vector containing the Ad35 fiber, pSQ1Ad35Fiber was digested with Clal and co-transfected into 633 cells with pAdmireRSVnBg which had been digested with Sall and Pacl. After propagation on 633 cells, the resulting 25 virus contained Ad5 fiber and Ad35 fibers on its capsid. The virus was amplified on PerC6 cells to generate virus containing only the Ad35 fiber on its capsid. The resulting virus preparation was termed Av1nBg35F.

Construction of adenoviral vectors containing chimeric fibers derived from Ad5 and Ad35: Two chimeric fiber constructs were prepared

by PCR gene overlap extension using plasmids containing the full length Ad5 or Ad35 fiber cDNAs as templates. The Ad5 fiber tail and shaft regions (5TS; amino acids 1 to 403) were connected with the Ad35 fiber head region (35H; amino acids 137 to 323) to form the 5TS35H chimera, and the Ad35 fiber tail and shaft regions (35TS; amino acids 1 to 136) were connected with the Ad5 fiber head region (5H; amino acids 404 to 581) to form the 35TS5H chimera. The fusions were made at the conserved TLWT sequence at the fiber shaft-head junction.

plasmid was used as the template with primers P1 and P2 to generate the 5' fragment. The 3' fragment was generated using the pFBshuttleAd35 plasmid as the template with the P3 and P4 primers. The sequence of each primer used in the construction of these chimeric fibers is listed in Table 2. Amplified PCR products of the expected size were obtained and were gel purified. A second PCR was carried out with the end primers P1 and P4 to join the two fragments together. The DNA fragment generated in the second PCR was digested with Xba1 and Mun1 and was cloned directly into pFBshuttle(EcoR1) to create the fiber shuttle plasmid pFBshuttle5TS35H.

TABLE 2
Primers Used For The Exchange Of Fiber Shaft Regions Between Ad5 And
Ad35 Fibers

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| Primer designation | Sequence | SEQ ID |
|--|--|--------|
| P1 | 5'-GAACAGGAGGTGAGCTTAGA-3' | 70 |
| P2 | 5'-GTTAGGTGGAGGGTTTATTCCGGTCCAC AAAGTTAGCTTATC-3' | 71 |
| Р3 | 5'-GATAAGCTAACTTTGTGGACCGGAATAAA CCCTCCACCTAAC-3' | 72 |
| P4 | 5'-GTGGCAGGTTGAATACTAGG-3 | 73 |
| P5 5'-GTTAGGAGATGGAGCTGGTGTAGTCCATA AGGTGTTAATAC-3' | | 74 |

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| Primer designation | Sequence | SEQ ID |
|--------------------|--|--------|
| P6 | 5'-GTATTAACACCTTATGGACTACACCAGCT CCATCTCCTAAC-3' | 75 |
| P7 | 5'-TGCGCAAAAACAATCACCACGACAATCACAAT GTACATTGGAAGAAATCATACG-3' | 76 |
| P8 | 5'-ACATTGTGATTGTCGTGGTGATT GTTTTTGCGCATATGCCATACAATTTGAATG-3' | 77 |

For the construction of the 35TS5H chimera, the pFBshuttleAd35 plasmid was used as the template with the P1 and P5 primers to generate the 5' fragment. The 3' fragment was generated using the pFBshuttle(EcoR1) plasmid as the template with the P6 and P4 primers. Following the same procedure described above, the fiber shuttle plasmid pFBshuttle35TS5H was generated.

For the 35TS5H and 5TS35H chimeras, the fiber gene was transferred from the pFBshuttle(EcoRI) backbone into pSQ1 as described above for the vector containing the Ad35 fiber. The resulting plasmids were called pSQ135T5H (Figure 18A) and pSQ15T35H (Figure 18B). In addition, adenoviral vectors were generated using the co-transfection strategy described above.

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Construction of Ad5 vectors containing the Ad35 fiber with a cRGD targeting peptide in the HI loop of the 35F fiber knob: To incorporate the cRGD targeting peptide into the Ad35 fiber HI loop, the P7 and P8 oligonucleotide primers encoding the ten amino acid sequence HCDCRGDCFC (SEQ ID NO. 78) were synthesized. The pFBshuttleAd35 plasmid containing the full length Ad35 fiber cDNA was used as the template in the PCR reaction with the P1 and P7 primer pair or with the P4 and P8 primer pair in order to generate the 5' and 3' PCR fragments.

25 A second PCR was then carried out with the end primers P1 and P4 to join the two fragments together. The resulting PCR fragment was

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digested with Xba1 and Mun1 and was cloned into pFBshuttle(EcoR1) to create the fiber shuttle plasmid pFBshuttleAd35cRGD. The modified Ad35 fiber gene was transferred into pSQ1 using the EcoRI cloning strategy described above to generate pSQ1Ad35FcRGD (Figure 17B). Adenoviral vector was generated using the co-transfection strategy

EXAMPLE 10

described above.

In Vitro Evaluation Of Adenoviral Vectors Containing 35F And Derivatives Thereof

10 The transduction efficiencies of adenoviral vectors containing the 35F or derivatives thereof were evaluated on A549 cells. The transduction efficiencies were compared to that of Av1nBg, an adenoviral vector containing the 5F fiber. The day prior to infection, cells were seeded into 24-well plates at a density of approximately 1 x 10⁵ cells per 15 well. Immediately prior to infection, the exact number of cells per well was determined by counting a representative well of cells. Each of the vectors, Av1nBg, Av1nBg35F, Av1nBg5T35H and Av1nBg35T5H were used to transduce A549 cells from 0 up to 1,000 particle per cell (PPC) ratios. The cell monolayers were stained with X-gal 24 hours after 20 infection and the percentage of cells expressing eta-galactosidase was determined by microscopic observation and counting of cells. Transductions were done in triplicate and three random fields in each well were counted, for a total of nine fields per vector. The results (Figure 19) showed similar transduction efficiencies on A549 cells using the Av1nBg35F and Av1nBg5T35H vectors compared to Av1nBg. The 25 Av1nBg35T5H showed much lower transduction efficiencies on A549 cells compared to Av1nBg as a result of the Ad35 shaft domain. The Ad35 shaft domain does not contain a HSP binding motif and the Av1nBg35T5H vector behaves similarly to the Av1nBgS* vector in vitro

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and *in vivo*. These studies also demonstrate that vectors containing fiber proteins without an HSP binding site are fully viable.

EXAMPLE 11

in Vivo Evaluation Of Adenoviral Vectors Containing 35F And Derivatives5 Thereof

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The objective of this study was to evaluate the in vivo biodistribution of adenoviral vectors containing 35F fibers and derivatives thereof to determine whether vectors containing these fibers ablate liver transduction due to their shaft regions. A positive control cohort received Av1nBg and a negative control group received HBSS. Cohorts of five C57BL/6 mice received each vector via tail vein injection at a dose of 1 x 10¹³ particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. The median lobe of the liver was placed in neutral buffered formalin to preserve the sample for β -galactosidase immunohistochemistry. In addition, tissue from each organ was frozen to preserve it for hexon PCR analysis to determine vector content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. β galactosidase immunohistochemistry, hexon real-time PCR and the chemiluminescent β -galactosidase activity assay were carried out as described in example 3.

The results of the β -galactosidase activity assay showed a dramatic reduction in liver transduction by vectors containing the Ad35 fiber or the 35T5H derivative (Figure 20) with an approximately 4- to 24-fold reduction in β -galactosidase activity in the liver compared to the control vector Av1nBg. These data demonstrate that shaft domains without HSP binding sites can effectively ablate hepatic *in vivo* gene transfer. In

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particular, HSP is the major entry mechanism for liver *in vivo*. CAR binding is a minor entry pathway.

EXAMPLE 12

Construction Of Ad5 Vectors Containing The Ad Serotype 41 Short Fiber 5 And Derivatives Thereof

The human adenovirus serotype 41 contains two different fibers on its capsid, encoded by two adjacent genes. One fiber has a molecular weight of 60kDa and is approximately 315A in length and is termed the long fiber. The other fiber has a molecular weight of 40kDa and is approximately 250— in length and is termed the short fiber. The Ad41 short fiber does not bind CAR and does not possess the heparin binding domain (KKTK) in its shaft. Therefore, this fiber provides a useful platform for adenoviral vector targeting.

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Construction of adenoviral vectors based on Ad5 but containing the Ad41 short fiber: A PCR SOEing strategy was used to generate a vector 15 based on the Ad5 genome but containing the Ad41 short (Ad41s) fiber. First, PCR was used to amplify the region of pSQ1 between the Xbal site at bp 25,309 and the start of the fiber gene. The primer pair used for the PCR were P-0005/U and P-0010/L. The DNA sequence of P-0005/U was as follows: 5' C TCT AGA AAT GGA CGG AAT TAT TAC AG 3' (SEQ ID 20 NO. 65). The sequence corresponds to bp 25,308 through 25,334 of pSQ1 and overlaps the Xbal site in that region. The DNA sequence of P-0010/L was as follows: 5' TTC TTT TCA T CTG CAA CAA CAT GAA GAT AGT G 3' (SEQ ID NO. 79). It contains a 5' extension corresponding to the first 10 bp of the Ad41s fiber gene. The remainder 25 of the primer anneals to pSQ1 immediately 5' of the ATG start codon of the fiber gene. The PCR product was the expected size (583 bp). A second PCR was used to amplify the Ad41s fiber using the plasmid pDV60Ad41sF as a template. The primers used were P-0011/U and

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P-0012/L. The DNA sequence of P-0011/U was as follows: 5' GT TGT TGC AG ATG AAA AGA ACC AGA ATT GAA G 3' (SEQ ID NO. 80). It contains a 10 bp 5' extension corresponding to the DNA sequence immediately 5' of the ATG start codon of the fiber gene in pSQ1. The remainder of the primer anneals to the beginning of the Ad41s fiber gene in pDV60Ad41sF. The DNA sequence of P-0012/L was as follows: 5' TG CAA TTG AAA AAT AAA CAC GTT GAA ACA TAA CAC AAA CGA TTC TTT ATT C TTC AGT TAT GTA GCA AAA TAC A 3' (SEQ ID NO. 81). It contains a 51 bp 5' extension corresponding to the sequence in pSQ1 10 from the last codon of the fiber gene through the Munl site 40 bp downstream of the fiber gene. The remainder of the primer anneals to the 3' end of the Ad41s fiber gene in pDV60Ad41sF. The PCR product was the expected size (1219 bp). The two PCR products were joined by PCR SOEing using primers P-0005/U and P-0009/L. The DNA sequence of 15 P-0009/L was described above. The PCR SOEing reaction yielded the expected 1782 bp product. The product was cloned into pCR4blunt-TOPO to yield pCR4blunt-TOPOAd41sF. Next, pCR4blunt-TOPOAd41sF was digested with Xbal and Munl and the 1773 bp fragment containing the Ad41s fiber gene was gel purified. This 20 fragment was ligated with the 6477 bp Xbal to Munl fragment of pFBshuttle(EcoRI) to generate pFBshuttleAd41sF. The Ad41s fiber gene was transferred into the pSQ1 backbone as follows. First, pFBshuttleAd41sF was linearized using EcoRI and this fragment was ligated with the 24,213 bp EcoRI fragment of pSQ1 to generate 25 pSQ1Ad41sF (Figure 21A). Adenoviral vector containing the Ad41s fiber was generated using the co-transfection strategy described above.

Construction of Ad5 adenoviral vectors containing the Ad41 short fiber with a cRGD targeting ligand in the HI loop: A PCR SOEing strategy was used to generate a construct containing the Ad41s fiber with cRGD

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in the HI loop. The plasmid pFBshuttleAd41sF was used as a template for the PCR amplifications. First, a 1782 bp fragment was amplified using primers 5FF and 41sRGDR. The primer 5FF was described above. It anneals to pFBshuttleAd41sF at the Xbal site upstream of the fiber gene. The DNA sequence of the primer 41sRGDR was as follows: 5' AGT ACA AAA ACA ATC ACC ACG ACA ATC ACA GTT TAT CTC GTT GTA GAC GAC ACT GA 3' SEQ ID NO. 82). It contains a 30 bp 5' extension that encodes the cRGD targeting ligand. The remainder of the primer anneals to pFBshuttleAd41sF from bp 2878 through 2903. A second PCR amplified a 277bp region of pFBshuttleAd41sF using primers 3FR and 41sRGDF. The primer 3FR was described previously. It anneals to pFBshuttleAd41sF at the MunI site downstream of the fiber gene. The DNA sequence of 41sRGDF was as follows: 5' TGT GAT TGT CGT GGT GAT TGT TTT TGT ACT AGT GGG TAT GCT TTT ACT TTT 3' (SEQ ID NO. 83). It contains a 30 bp 5' extension that encodes the cRGD targeting ligand and is complementary to the extension on 41sRGDR. The remainder of the primer anneals to pFBshuttleAd41sF from bp 2904 through 2924. The two PCR products were joined by PCR SOEing to generate a 2059 bp fragment using primers 5FF and 3FR. The product was digested with Xbal and Munl and the 1803 bp DNA fragment was gel purified. The fragment was ligated with the 6477 bp fragment resulting from digestion of pFBshuttle(EcoRI) with Xbal and Munl. The resulting plasmid was termed pFBshuttleAd41sRGD. This plasmid was linearized by EcoRl digestion and ligated with the 24,213bp EcoRl

fragment of pSQ1 to generate pSQ1Ad41sRGD (Figure 21B).

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EXAMPLE 13

In Vivo Evaluation Of Ad5 Vectors Containing The Ad41 Short Fiber And Derivatives Thereof

This example evaluates the *in vivo* biodistribution of adenoviral vectors containing 41sF fibers and derivatives thereof to determine whether vectors containing the these fibers ablate liver transduction due to modified shaft regions. A positive control cohort received Av3nBg (see, Gorziglia *et al.* (1996) *J. Virology 70*:4173-4178) or Ad5.βGal.ΔF/5F, and a negative control group received HBSS.

Ad5.βGal.ΔF/5F is a derivative of the fiberless vector Ad5.βgal.ΔF (ATCC accession number VR2636) modified to express AD5 fiber (see, e.g., International PCT application No. WO 01/83729).

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The Ad5. β Gal. Δ F vector was pseudotyped with the Ad41sF fiber protein and injected *in vivo*. Cohorts of five C57BL/6 mice received each vector via tail vein injection at a dose of 1 x 10¹³ particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. The median lobe of the liver was placed in neutral buffered formalin to preserve the sample for β -galactosidase immunohistochemistry. In addition, tissue from each organ was frozen to preserve it for hexon PCR analysis to determine vector content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. β -galactosidase immunohistochemistry, hexon real-time PCR and the chemiluminescent β -galactosidase activity assay was carried out as described in Example 3.

The results of the hexon DNA analysis showed a dramatic reduction in liver transduction by vectors containing the Ad41sF fiber

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(Figure 22) with an approximately a 5-fold reduction in liver adenoviral DNA content compared to either control vector.

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In the above examples, several novel adenoviral vectors were generated containing various fiber modifications designed to ablate the normal tropism of the vector (see Table 3). Vectors were generated in which the heparan sulfate binding domain in the fiber shaft was replaced by amino acid substitutions. This mutation, termed HSP, was also combined with the KO1 mutation (fiber knob AB loop mutation that ablates CAR binding), and the PD1 mutation (penton mutation that eliminates RGD/integrin interaction). In addition, a vector containing all three mutations (HSP, KO1, PD1) was generated. All vectors containing the HSP mutation, either alone or combined with other capsid modifications, showed dramatically reduced transduction efficiencies on A549 and HeLa cells. Furthermore, the same vectors showed dramatically reduced transduction of the liver following systemic delivery to mice. As an alternative strategy to ablate the normal tropism of Ad5-based vectors, the Ad5 fiber was replaced by a fiber from a different adenovirus serotype which does not bind CAR and does not contain the heparan binding domain in the shaft. Thus, vectors were generated containing the Ad35 fiber and the Ad41 short fiber. Versions of these two vectors containing a cRGD targeting ligand in the HI loop of the fiber were also produced. Additionally, vectors containing chimeric fibers were generated. A vector containing the Ad35 fiber tail and shaft regions fused to the Ad5 fiber knob domain as well as a vector containing the Ad5 fiber tail and shaft fused to the Ad35 fiber knob domain were constructed. Vectors containing either the entire Ad35 or Ad41 short fiber showed a significant reduction in liver transduction following delivery to mice via the tail vein. The observation of reduced liver transduction using vectors containing either an HSP mutation, the Ad35 fiber, or the

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Ad41 short fiber indicates the feasibility of detargeting adenoviral vectors in vivo. In vitro data with the Ad35 fiber or the Ad41 short fiber with cRGD (see Example 14) indicate that the virus is completely viable, that is, it is not damaged by the absence of an HSP binding site and is retargetable. Taken together these data suggest that these vectors provide a suitable platform for retargeting strategies.

TABLE 3
Description Of Recombinant Adenoviral Vectors Used
To Demonstrate That Shaft Modifications Influence Tropism *In Vivo*

10 Vector Vector Description Av1nBg An E1 and E3-deleted adenoviral vector encoding a nuclear localizing β -galactosidase Ad5 Fiber derivatives: Av1nBgFKO1 The same as Av1nBg but containing the KO1 AB loop mutation in the fiber gene 15 Av1nBgPD1 The same as Av1nBg but containing the penton PD1 mutation that deletes the integrin binding, RGD tripeptide Av1nBgS* The same as Av1nBg but containing the 4 amino acid substitution in the shaft referred to as S* that modifies the HSP binding motif Av1nBgFKO1S* The same as Av1nBg but containing the fiber KO1 and S* mutations combined Av1nBgS*PD1 The same as Av1nBg but containing the fiber S* and penton PD1 mutations combined Av1nBgFKO1S*PD1 The same as Av1nBg but containing the fiber KO1, S* and penton PD1 mutations combined 20 Ad35 fiber derivatives: Av1nBg35F The same as Av1nBg but containing the full length Ad35 fiber Av1nBg5T35H The same as Av1nBg but containing the 5T35H chimeric fiber Av1nBg35T5H The same as Av1nBg but containing the 35T5H chimeric fiber Av1nBg35FRGD The same as Av1nBg but containing the full length Ad35 fiber cDNA with a cRGD ligand in the HI loop of the Ad35 fiber 25 Ad41sF fiber derivatives:

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| Vector | Description | | |
|---------------|---|--|--|
| Av1nBg41sF | The same as Av1nBg but containing the full length Ad41 short fiber cDNA | | |
| Av1nBg41sFRGD | The same as Av1nBg but containing the full length Ad41 short fiber cDNA with a cRGD ligand in the HI loop of the Ad41 short fiber | | |

EXAMPLE 14

5 In Vitro Evaluation Of Adenoviral Vectors Containing The Ad41sF With A cRGD Ligand In The HI Loop

The transduction efficiencies of adenoviral vectors containing the Ad41sF fiber with the cRGD ligand in the HI loop were evaluated on A549 cells. The transduction efficiencies were compared to that of 10 Av1nBg, an adenoviral vector containing wild type fiber or Av1nBgFKO1RGD, an adenoviral vector containing the KO1 mutation in combination with the cRGD ligand in the HI loop. The day prior to infection, cells were seeded into 24-well plates at a density of approximately 1 x 10⁵ cells per well. Immediately prior to infection, the 15 exact number of cells per well was determined by counting a representative well of cells. Each of the vectors, Av1nBg, Av1nBgFKO1RGD, and Av1nBg41sFRGD were used to transduce A549 cells at a particle to cell ratios of 0 up to 10,000. The cell monolayers were stained with X-gal 24 hours after infection and the percentage of 20 cells expressing β -galactosidase was determined by microscopic observation and counting of cells. Transductions were done in triplicate and three random fields in each well were counted, for a total of nine fields per vector. The results (Figure 23) show that the Av1nBg41sFRGD vector transduced cells to an equivalent level as Av1nBgFKO1RGD at all 25 vector doses examined. Neither FKO1 or Ad41sF can bind CAR. The Ad41sF does not normally interact with CAR and additionally does not contain the HSP binding motif within the shaft domain. These data show

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that targeting peptides inserted into the loop regions of the fiber knob of KO1 and Ad41sF allows for transduction of target cells via the targeted receptor. Surprisingly, HSP, not CAR and integrins, is the major entry route *in vivo* and ablation of HSP binding permits targeting of adenoviral vectors.

EXAMPLE 15

Effect of the shaft modification on the biodistribution of adenoviral vectors in vivo

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The influence of fiber and penton modifications on the in vivo biodistribution of adenoviral vectors containing fiber head, shaft and 10 penton mutations was examined. Vectors containing the HSP mutation combined with KO1, or PD1, or a combination of all three mutations were evaluated as well as vectors containing the KO1 mutation alone and the PD1 mutation alone. The indicated adenoviral vectors were systemically administered to C57BL6 mice as described above. A positive control 15 cohort received Av1nBg and a negative control group received HBSS. Cohorts of five C57BL/6 mice received each vector via tail vein injection at a dose of 1 \times 10 13 particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from 20 each animal. Tissue from each organ was frozen to preserve it for real time PCR analysis to determine adenoviral hexon DNA content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent eta-galactosidase activity assay. Hexon real-time PCR and the chemiluminescent β -galactosidase activity assay was carried out 25 as described in Example 3.

The results derived from the liver are described in Example 6 (Figure 14A and B) and also shown in Figure 26 with results presented as percent control of Av1nBg. The effect of the S* shaft modification on

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the biodistribution of adenovirus to the other organs is shown in Figure 25. The average adenoviral DNA content was determined as adenoviral genomic copies per cell and expressed as a percentage of the Av1nBg (+) control value. The average percent control value + standard deviation is shown (n = 5 per group) for each tissue examined (Figure 25).

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Systemic delivery of Ad5 based vectors with wild-type fiber results in a preferential accumulation of vector DNA in the liver with 64 copies per cell with significantly less DNA found in the other organs with 1.32 copies per cell found in lung, 2.18 copies per cell in spleen, 0.47 copies per cell found in heart, and 0.72 copies per cell in the kidney. All differences found with PD1, S*, KO1PD1, KO1S*, S*PD1, and KO1S*PD1 were significantly different than the Av1nBg (+) control using a unpaired, t-test analysis, P value (0.024. When expressed as a percent of the Av1nBg control values, the influence of each mutation, individually or in combination, becomes apparent. The S* mutation dramatically reduced gene transfer to all four organs, whereas, the KO1 mutation did not. Thus, the importance of the shaft for transduction *in vivo* extends to organs besides the liver. Finally, gene transfer to the lung, heart, and kidney was diminished with PD1 suggesting a role for integrin binding in vector entry in these organs.

EXAMPLE 16

Retargeting the S*, shaft modification and the 41sF fiber in vivo

Vectors containing the HSP mutation have been shown to effectively detarget adenoviral vectors *in vivo* (see examples 6 and 15). The objective of this study was to evaluate the ability to retarget vectors containing the S* modification or the Ad41sF to tumors *in vivo*. A cRGD peptide was genetically incorporated into the fiber HI loop and evaluated *in vitro* (Examples 8 and 14). These same vectors were then evaluated *in vivo* in tumor-bearing mice. Athymic nu/nu female mice were injected

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with 8 x 10^6 A549 cells on the right hind flank. When tumors reached approximately 100mm3 in size, they were randomized into treatment groups. Cohorts of 6 mice received each vector via tail vein injection at a dose of 1 x 10^{13} particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Tumor, liver, heart, lung, spleen, and kidney were collected from each animal. Tissue from each organ was frozen to preserve it for real time PCR analysis to determine adenoviral hexon DNA content. Hexon real-time PCR was carried out as described in example 3. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. Hexon real-time PCR and the chemiluminescent β -galactosidase activity assay was carried out as described in example 3.

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The adenoviral vector biodistribution to the liver and tumor for each treatment group is shown in Figure 27. Vectors containing the S*, KO1S*, and 41sF fibers effectively detargeted the liver and tumor resulting in a significant reduction in the amount of adenoviral DNA found in each tissue in comparison to the Av1nBg control. Vectors containing the cRGD targeting ligand restored tranduction of the tumors to levels comparable to that achieved with the untargeted vector.

These data demonstrate successful liver detargeting accompanied with tumor retargeting. The extent of tumor retargeting is relates to the affinity and type of ligand that is used. These data demonstrate the successful development of a targeted, systemically deliverable adenoviral vector that will target tumors *in vivo*.

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EXAMPLE 17

Scale-Up Method For The Propagation Of Detargeted Adenoviral Vectors

The growth and propagation of doubly or triply ablated adenoviral vectors requires novel scale up technologies. These detargeted vectors require alternative cellular entry strategies to allow for the efficient growth and generation of high titer preparations. A strategy for vector growth that is generally applicable to all detargeted adenoviral vectors, that does not require the development of new cell lines, and that aslo can be used for generating targeted vectors is provided herein.

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Three recombinant adenoviral vectors were prepared that contain single mutations in the fiber or penton or both mutations combined into one vector. These vectors are designated Av3nBgFKO1, Av1nBgPD1, and Av1nBgFKO1PD1, respectively. The construction of these vectors is described above and a general description of each vector can be found in Table 1 above.

Scale-up of detargeted adenoviral vectors: A polycation, specifically hexadimethrine bromide was obtained from Sigma Chemical Co (St. Louis, MO), Catalog No. 52495, and was maintained in the medium at 4 μ g/ml during the course of transfections and infections. To illustrate the affects of hexadimethrine bromide on the yield of detargeted adenoviral vectors the following experiment was carried out. Seven plates of AE1-2a adenoviral producer cells (Gorziglia *et al.* (1996) *J. Virology* 70:4173-4178) were transduced with 10 particles per cells of each of the indicated vectors (See Table 4). Each vector was incubated with medium (Richters with 2% HI-FBS) containing hexadimethrine bromide at 4 μ g/ml for 30 min at room temperature prior to infection. The infection was carried out for 2 hrs. Complete medium containing hexadimethrine bromide at 4 μ g/ml was added to each plate. Final concentration of hexadimethrine bromide in all of these experiments was maintained at

4 μ g/ml. The titers were determined spectrophotometrically using the conversion of 10D at A260nm per 1 x 10¹² particles (Mittereder *et al.* (1996) *J Virology 70*:7498-7509). The total particle yield was then normalized for the number of plates used for transduction.

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The inclusion of hexadimethrine bromide in the medium during the course of infection allows for the efficient propagation of detargeted adenoviral vectors containing fiber and penton mutations either alone or in combination. The effect of hexadimethrine bromide on vector yields is shown in Table 4. A 35-fold improvement in the yield of Av3nBgFKO1 was found when hexadimethrine bromide was included in the culture medium and resulted in increasing the vector yield from 1.3 x 10¹⁰ up to 4.6 x 10¹¹ vector particle per plate. Hexadimethrine bromide has a minimal effect on the yield of the Av1nBgPD1 adenoviral vector containing the penton, PD1 mutation with only a 1.2 fold improvement.

The greatest effect using hexadimethrine bromide was found on the propagation of the doubly ablated adenoviral vector, Av1nBgFKO1PD1 with increases in vector yield from barely detectable levels up to 4.53 x 10¹⁰ vector particles per plate. These data demonstrate that use of nonspecific entry mechanisms allows for the efficient scale-up of detargeted adenoviral vectors.

TABLE 4
Efficient Scale-Up Of Detargeted Adenoviral Vectors Using hexadimethrine bromide

| | Vector Yield (particles/plate) | | |
|------------|--------------------------------|-------------------------------|---------------------|
| Vector | (-) hexadimethrine bromide | (+) hexadimethrine bromide | Fold Improvement |
| Av1nBg | 3.89 x 10 ¹¹ | 5.72 x 10 ¹¹ | 1.47 |
| Av3nBg | 8.58 x 10 ¹⁰ | 2.38 x 10 ¹¹ | 2.77 |
| Av3nBgFKO1 | 1.30 x 10 ¹⁰ | 4.60 x 10 ¹¹ | 35.4 |
| Av1nBgPD1 | 1.95 x 10 ¹¹ | 2.40 x 10 ¹¹ | 1.23 |

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| | Vector Yield (| | |
|---------------|-------------------------------|-------------------------------|------------------|
| Vector | (-) hexadimethrine bromide | (+) hexadimethrine bromide | Fold Improvement |
| Av1nBgFKO1PD1 | TLTC* | 4.53 x 10 ¹⁰ | Ť |

^{*}TLTC: Too low to count, a faint virus band was collected and the particle concentration was too dilute for titer determination.

[†] Significant improvement

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The use of alternative polycations including protamine sulfate and poly-lysine as well as bifunctional proteins such as the anti-penton: $TNF\alpha$ fusion protein was investigated. Figure 24 show results that demonstrate all the reagents tested had some effect on enhancing transduction of the Av3nBgFKO1 vector. All of these compounds, when maintained in the medium during infection, enhanced transduction of the Av3nBgFKO1 detargeted adenoviral vector.

Bifunctional reagents:. The use of bifunctional reagents for the propagation of detargeted adenoviral vectors was examined using the anti-penton:TNF α fusion protein. This particular reagent is a fusion protein between an antibody against Ad5 penton and the TNF α protein that is produced using stably transfected insect cells. This reagent will bind specifically to the adenoviral capsid via penton base and allow for binding to cell surface TNF receptors. The use of this reagent for the propagation of detargeted vectors is illustrated in Table 5 using Av3nBgFKO1 (also shown in Figure 24). Monolayers of S8 cells were infected with 10 or 100 particles per cell of Av3nBgFKO1 or a control vector in the presence or absence of 1 μ g/ml of the anti-penton:TNF α fusion protein. The monolayers were visually inspected over time for vector spread as indicated by the extent of cytopathic effect (CPE). The percentage of CPE at each time point is shown. The use of this bifunctional reagent

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clearly enhances the spread of the Av3nBgFKO1 vector throughout the monolayer.

TABLE 5
Efficient Scale-Up Of Detargeted Adenoviral
Vectors Using Bifunctional Reagents: Anti-Penton:TNFa

| | | 10 ppc - anti-penton TNF | 10 ppc + anti-penton TNF | 100 ppc - anti-penton TNF | 100 ppc + anti-penton TNF | | |
|----|---------|--------------------------------|--------------------------------|------------------------------|------------------------------|--|--|
| | | | Percentage of CPE | | | | |
| | Ad5Luc1 | | | | | | |
| 10 | 24 h | 0% | 0% | 0% | 0% | | |
| | 48 h | 20-30% | 20-30% | 90-100% | 90-100% | | |
| | 72 h | 60-70% | 80-90% | 100% | 100% | | |
| | 120 h | 100% | 100% | 100% | 100% | | |
| | Av3nBgk | (O1 24hrs | | | | | |
| 15 | 24 h | 0% | 0% | 0% | 0% | | |
| | 48 h | 0% | 10-20% | 0% | 90-100% | | |
| | 72 h | 5% | 60-70% | 5% | 100% | | |
| | 120 h | 40-50% | 100% | 100% | 100% | | |

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EXAMPLE 18

This Example and the following Example describe construction of adenoviral Ad5 particles that express heterologous fibers. The fibers are modified at the N-terminus to increase incorporation into the Ad5 particle. The N-terminus, typically, the first at least 1.6 or 17 amino acids is modified so that the sequence resembles the Ad5 terminus.

Expression of Fiber Proteins from Subgroups B, C and D of Human Adenovirus

Constructs for expression of fibers from several adenoviral serotypes including, Types 16, 30, and 35 (subgroup B), and Types 19p and 37 (subgroup D), were generated.

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Construction of Ad37, Ad19p and Ad30 fiber expression plasmids

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The open reading frames (ORFs) of Ad37 (SEQ ID NO. 31), Ad19p (SEQ ID NO. 33) or Ad30 (SEQ ID NO. 35) fiber proteins were PCR amplified using the following primers:

Forward primer (L37): TGT CTT **GGA TCC** AAG ATG AAG CGC GCC CGC CCC AGC GAA GAT GAC TTC (SEQ ID NO. 84)

Reverse primer (37FR): AAA CAC G**GC GGC CGC** TCT TTC ATT CTT G (SEQ ID NO. 85)

Primers L37 and 37FR include BamHI and NotI sites (in bold), respectively, to facilitate subcloning. In addition, primer L37 introduces mutations into the 5' end of each fiber protein so that the resulting fiber proteins more closely resemble the Ad5 fiber N-terminal sequence for assembly onto Ad5 particles. For Ad37, the native N-terminus and modified N-terminus have the following amino acid sequences:

Native Ad37 N-terminus: MSKRLRVE (SEQ ID NO. 86)

Modified Ad37 N-terminus: MKRARPSE (SEQ ID NO. 87)

The amplified fibers were cloned into the BamHI and Notl sites of plasmid pCDNA3.1zeo(+) (Invitrogen). The Ad5 tripartite leader (TPL) sequence from plasmid pDV55 (see EXAMPLE 20 and SEQ ID NO. 88), which is flanked by BamHI sites (SEQ ID NO. 88), was then subcloned into the BamHI site of each fiber expression plasmid to generate plasmids pDV121 (Ad37), pDV145 (Ad19p) and pDV164 (Ad30). Construction of plasmid pDV55 is set forth in EXAMPLE 20 (see also, copending U.S. application Serial No. 09/482,682, also filed as International PCT application No. PCT/US00/00265; and in U.S. application Serial No. 09/562,934, also filed as International PCT application No. PCT/EP01/04863. The combination of the CMV promoter present in pCDNA3.1zeo(+) and the addition of the TPL sequence from pDV55

(SEQ ID NO. 88) provides for high-level expression of viral proteins.

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Construction of Ad16 and Ad35 fiber expression plasmids

Ad16 and Ad35 fiber expression plasmids were generated in a similar manner with the following modifications. To PCR amplify Ad16 (SEQ ID NO. 37) and Ad35 (SEQ ID NO. 39) fiber, the forward primer was designed to incorporate an Ndel site (in bold), which is present at nucleotide 48 of Ad5 fiber (SEQ ID NO. 1), but absent in Ad16 and Ad35 fiber sequences. The reverse primers contained a Notl site (in bold):

Ad16/Ad35 forward primer (F16 5'): CCG GTC TAC CCA TAT GAA GATG (SEQ ID NO. 89)

Ad16 reverse primer (F16 3'): TGG TGC GGC CGC TCA GTC ATC TTC TCTG (SEQ ID NO. 90)

Ad35 reverse primer (F35 3'): TGG TGC GGC CGC TTA GTT GTC GTC TTC TGT AAT G (SEQ ID NO. 91)

The Ad16 and Ad35 PCR products were cloned into the Ndel and 15 Notl sites of pCDNA3.1zeo(+), resulting in plasmids pDV147 (Ad16) and pDV165 (Ad35). The Ndel site of pCDNA3.1zeo(+) is within the CMV promoter region of the plasmid, therefore, the resulting plasmids lacked the 3' portion of the CMV promoter region. In addition, the inserted fiber sequences were lacking the portion of the fiber sequence that is 5' to the 20 engineered Ndel site. To insert the necessary regulatory sequences and N-terminal fiber sequence, plasmid pDV67 (described in Example 22 and also U.S. Application Serial No. 09/562,934, and is available from the ATCC under accession number PTA-1145) was digested with Ndel to remove a fragment that contains the 3' portion of the CMV promoter, the 25 complete Ad5 TPL sequence and the 5' portion of the Ad5 fiber sequence. The Ndel fragment was subcloned into plasmids pDV147 and pDV165 to generate the complete Ad16 and Ad35 expression plasmids, pDV156 and pDV166, respectively. Expression of these constructs results in chimeric fiber proteins containing the 17 N-terminal amino acids

from Ad5 fiber (see SEQ ID NO. 2) and the remainder of the fiber sequence from either Ad16 or Ad35. The nucleotide sequences of the chimeric fibers are listed in SEQ ID NO. 41 (Ad5/Ad16) and SEQ ID NO. 43 (Ad5/Ad35).

Expression and trimerization of recombinant Ad fiber proteins

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To verify expression and trimerization of the recombinant proteins, the resulting plasmids were transfected into 293T cells, which are identical to 293 cells except they express an integrated SV40 large T antigen gene. 239 cells are an adenovirus-transformed human embryonic kidney cell line obtained from the ATCC, where they are deposited under Accession Number CRL 1573. 293T cells express CAR and $a_{\rm v}$ integrins. Fiber expression was detected by immunoblotting of cell lysates using the 4D2 monoclonal antibody (Research Diagnostics Inc., Flanders, N.J.), which recognizes an epitope conserved among fibers of different serotypes. To generate stable cell lines, constructs were electroporated into an A549-derived cell line that complements the Ad E1a and E2a functions (Gorziglia *et al.*, *J. Virol. 70*:4173-4178 (1996)) and stable clones were derived by selection with zeocin. Clones that expressed high levels of the fiber protein were identified by immunoblotting with the 4D2 antibody.

Generation of Adenovirus Particles Pseudotyped with Subgroup B, C or D Ad Fiber Protein

A system for producing Ad vector particles with different or modified fiber proteins, that therefore have altered tropism (pseudotyping) is known (see, e.g., Von Seggern et al., J. Virol. 74:354-362 (2000); Wu et al., Virology 279:78-89 (2001)). Briefly, an E1-deleted Ad vector is modified by further deletion of the fiber gene, such that the virus produces no fiber protein. Growth of the fiber-deleted viruses in

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packaging cells that express a fiber protein as well as complementing the E1 deletion allows generation of particles with any desired fiber.

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Packaging cell lines were generated by stably transfecting expression constructs for the fibers of interest (Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000)) into an A549-derived E1- and E2a-complementing cell line (Gorziglia *et al.*, *J. Virol.* 70:4173-4178 (1996)), and clones that expressed the fibers at high levels were selected. The resulting lines complement E1 and fiber deletions, and were used to propagate Ad5.GFP.ΔF, a fiber-deleted Ad5 vector with a GFP transgene in place of the deleted E1 sequences (Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000)). The particles produced by growth in the various cell lines are identical except for their fiber proteins. Viral particles were isolated by CsCl gradient centrifugation, and assayed for the presence of fiber by immunoblotting using monoclonal Ab 4D2. As a control for equal loading, the blot was re-probed with a polyclonal antibody against the Ad penton base protein. All recombinant fibers were capable of assembly onto Ad5 particles.

EXAMPLE 19

Construction and Propagation of Adenovirus Particles with Genomic Fiber 20 Substitutions

The fiber-deletion system described in Example 18 allows rapid evaluation of fiber proteins for their infectious properties. The resulting particles produced are less infectious than the corresponding first-generation vectors (Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000)). Therefore, viral backbones with the subgroup B, C or D fibers substituted in place of the Ad5 fiber gene were constructed. To facilitate construction of these vectors, the AdEasy system (see, U.S. Patent No. 5,922,576; see, also He *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95:2509-2514; the system is publicly available from the authors and

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other sources) was modified. This system includes a large plasmid (pAdEasy) that contains most of the Ad5 genome and smaller shuttle plasmids with the left end of the viral genome, including an E1 deletion and polylinker for insertion of transgenes. Recombination between pAdEasy and a shuttle plasmid in *E. coli* reconstitutes a full-length infectious Ad genome. All plasmids used were derivatives of pAdEasy1 with different fiber proteins substituted in place of the Ad5 fiber.

Construction of pDV153

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p5FloxHRF (SEQ ID NO. 92) contains the right end of the Ad5 genome with a Pacl site in place of the right ITR. There is a unique (naturally occurring in Ad5) Munl site approximately 30 nucleotides downstream of the fiber stop codon. To facilitate fiber substitutions, this site was moved to lie immediately downstream of the fiber ORF. This preserved the sequence around the fiber gene and its spacing relative to the other adenovirus genes.

The oligos MunITOP (AAT TGT GTT ATG TTT AAA CGT GTT TAT TTT TG; SEQ ID NO. 93) and MunIBOTTOM (AAT TCA AAA ATA AAC ACG TTT AAA CAT AAC AC; SEQ ID NO. 94) were annealed and ligated into the unique MunI site of p5FloxHRF to generate plasmid pDV153. Insertion of the oligo destroyed the original MunI site by changing one base at its 5' end, but resulted in insertion of a new MunI site that is 32 base pairs closer to the fiber ORF than the original MunI site.

Construction of an Ad vector with a chimeric Ad5/Ad37 fiber gene

To replace the Ad5 fiber sequence of pDV153 with Ad37 fiber, pDV153 was digested with SphI and MunI. This removed all but the N-terminal 183 nucleotides of Ad5 fiber (see SEQ ID NO. 1). Ad37 fiber was then PCR amplified using a 5' primer (F37 5'SphI) TAC CAA TGG CAT GCT ATC CCT CAA GG (SEQ ID NO. 95) that added a SphI site and

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a 3' primer (F37 3'EcoRI) AAA CAC GGG AAT TCG TCT TTC ATT C (SEQ ID NO. 96) that added an EcoRI restriction site. The 3' primer was designed to have an EcoRI site since the Ad37 fiber sequence contains a MunI restriction site. The nucleotide overhangs left by digestion with EcoRI and MunI are compatible, allowing the PCR products to be cloned into pDV153 digested with SphI and MunI. This resulted in expression of a chimeric Ad5/Ad37 fiber protein with the N-terminal 61 amino acids from Ad5 fiber (SEQ ID NO. 2) and the remainder of the protein from Ad37 (corresponding to amino acid 62 to the end of Ad37 fiber; SEQ ID NO. 32).

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After Ad37 fiber was ligated into pDV153, the Spel/Pacl fragment was used to replace the Spel/Pacl fragment of pAdEasy, resulting in plasmid pDV158. Plasmid pDV158 was then recombined with the shuttle plasmid pAdTrack, which contains a CMV-driven EGFP reporter gene (He et al., Proc. Natl. Acad. Sci. USA 95:2509-2514 (1998); U.S. Patent Serial No. 5,922,576). The resulting Ad vector (Ad5.GFP.37F) has the EGFP reporter at the site of the E1 deletion and the chimeric Ad5/Ad37 fiber gene in the viral chromosome, and infects cells via the Ad37 receptor rather than CAR. pDV158 can be readily used to create adenovirus particles with the same fiber protein but different transgenes.

Propagation of Ad5.GFP.37F in 633 cells

The Ad5.GFP.37F genome is infectious, and readily begins replicating as a virus. Since the 293 cells (ATCC Accession No. CRL 1573) normally used for Ad propagation do not express high levels of the Ad37 receptor, this virus does not efficiently propagate. To facilitate viral amplification, stocks of the virus were maintained in the 633 cell line (ATCC Accession No. PTA-1145), which expresses a wildtype Ad5 fiber protein (Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000)). The particles therefore contain the Ad5 fiber produced by the cells and the chimeric

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Ad5/Ad37 fiber protein encoded by the virus. The Ad5 fiber allows the virus to re-infect the cell lines used for viral growth. A final round of growth in 293 cells (which do not express a fiber protein) generates particles with only the vector-encoded Ad37 fiber. To assess Ad5 and Ad37 fiber content of Ad5.GFP.37F particles, viral particles produced in either 633 cells or 293 cells were immunoblotted with anti-fiber monoclonal Ab 4D2. 633-grown particles contained the Ad5 and Ad5/37 fibers, while virus produced in 293 cells contained only the Ad5/37 chimeric fiber. Particles of the first-generation Ad vector Ad5.βgal.wt, which contain only the wildtype Ad5 fiber (Wu *et al.*, *Virology 279*:78-89 (2001)), were included as a positive control. As a loading control, the same blot was re-probed with a polyclonal antibody against the viral penton base protein.

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Preparation of additional Ad5 genomes encoding heterologous fibers

These same procedures can be used to construct Ad5 genomes containing the 19p (SEQ ID NO. 33), 16 (SEQ ID NO. 37), 30 (SEQ ID NO. 35) and 35 (SEQ ID NO. 39) fibers. To improve incorporation of the fiber in the resulting particle, each fiber was modified to include the N-terminal 61 amino acids of Ad5 (see SEQ ID NO. 2 or see nucleotides 1-183 in SEQ ID NO. 1) by replacing the corresponding amino acids (*i.e.*, the first 61 amino acids) of each heterologous fiber. Similar constructs can be made with other heterologous fibers and genomes, such as Ad2.

For example, for construction of the Ad5/Ad16 chimeric fiber vector, plasmid pDV153 was digested with SphI and MunI to remove all but the first 183 nucleotides of Ad5 fiber. Ad16 fiber (SEQ ID NO. 37) was PCR amplified using 5' primer F16 5' SphI: GCC AGC GGC ATG CTC CAA CTT AAA (SEQ ID NO. 97) and 3' primer F16 3' MunI: TTT ATC AAT TGT GTT GTC AGT CAT CTT C (SEQ ID NO. 98), which contained

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SphI and MunI sites, respectively. The PCR product was ligated with plasmid pDV153 to generate plasmid pDV182. This resulted in expression of a chimeric Ad5/Ad16 fiber protein with the N-terminal 61 amino acids from Ad5 fiber (SEQ ID NO. 2) and the remainder of the protein from Ad16 (corresponding to amino acid 62 to the end of Ad16 fiber; SEQ ID NO. 38).

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EXAMPLE 20

Tripartite leader sequences (TPLs) that are useful in enhancing the expression of complementing adenoviral proteins, particularly fiber 10 protein, for use in preparing an adenoviral gene delivery vector are provided. The complete Ad5 TPL was constructed by assembling PCR fragments. First, the third TPL exon (exon 3) (nt 9644-9731 of the Ad5 genome) was amplified from Ad5 genomic DNA using the synthetic oligonucleotide primers 5'CTCAACAATTGTGGATCCGTACTCC3' (SEQ ID 15 NO. 99) and 5'GTGCTCAGCAGATCTTGCGACTGTG3' (SEQ ID NO. 100). The resulting product was cloned to the BamHI and BgIII sites of pΔE1Sp1a (Microbix Biosystems; see also, U.S. Patent No. 6,140,087 and U.S. Patent No. 6,379,943) using sites in the primers (shown in bold) to create plasmid pDV52. A fragment corresponding to the first TPL exon (exon 1), the natural first intron (intron 1), and the second TPL exon 20 (exon 2) (Ad5 nt 6049-7182) was then amplified using primers 5'GGCGCGTTCGGATCCACTCTCTTCC3' (SEQ ID NO. 101) and 5'CTACATGCTAGGCAGATCTCGTTCGGAG3' (SEQ ID NO. 102), and cloned into the BamHI site of pDV52 (again using sites in the primers) to 25 create pDV55.

This plasmid contains a 1.2 kb BamHI/BgIII fragment containing the first TPL exon, the natural first intron, and the fused second and third TPL exons. The nucleotide sequence of the complete TPL containing the noted 5' and 3' restriction sites is shown in SEQ ID NO. 103 with the

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following nucleotide regions identified: 1-6 nt BamHI site; 7-47 nt first leader segment (exon 1); 48-1068 nt natural first intron (intron 1); 1069-1140 nt second leader segment (exon 2); 1141-1146 nt fused BamHI and BgIII sites; 1147-1234 nt third leader segment (exon 3); and 1235-1240 nt BgIII site.

EXAMPLE 21

Preparation of Adenoviral Gene Delivery Vectors Using Adenoviral Packaging Cell Lines

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Adenoviral delivery vectors are prepared to separately lack the combinations of E1/fiber and E4/fiber. Such vectors are more replication-defective than those previously in use due to the absence of multiple viral genes. A preferred adenoviral delivery vector is replication competent but only via a non-fiber means is one that only lacks the fiber gene but contains the remaining functional adenoviral regulatory and structural genes. Furthermore, these adenovirus delivery vectors have a higher capacity for insertion of foreign DNA.

A. Preparation of Adenoviral Gene Delivery Vectors Having Specific Gene Deletions and Methods of Use

To construct an E1/fiber deleted viral vector containing the LacZ reporter gene construct, two new plasmids were constructed. The plasmid $p\Delta E1B\beta$ gal was constructed as follows. A DNA fragment containing the SV40 regulatory sequences and *E. coli* β -galactosidase gene was isolated from $pSV\beta$ gal (Promega) by digesting with VspI, filling the overhanging ends by treatment with Klenow fragment of DNA polymerase I in the presence of dNTPs and digesting with BamHI. The resulting fragment was cloned into the EcoRV and BamHI sites in the polylinker of p Δ E1sp1B (Microbix Biosystems; see also, U.S. Patent No. 6,140,087 and U.S. Patent No. 6,379,943) to form p Δ E1B β gal that therefore contained the left end of the adenovirus genome with the Ela region replaced by the LacZ cassette (nucleotides 6690 to 4151) of

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pSV β gal. Plasmid DNA may be prepared by the alkaline lysis method as described by Birnboim and Doly, *Nuc. Acids Res.*, 7:1513-1523 (1978) or by the Quiagen method according to the manufacturer's instruction, from transformed cells used to expand the plasmid DNA. Plasmid DNA was then purified by CsCl-ethidium bromide density gradient centrifugation. Alternatively, plasmid DNAs may be purified from *E. coli* by standard methods known in the art (e.g. see Sambrook et al.)

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The second plasmid (pDV44), prepared as described herein, is derived from pBHG10, a vector prepared as described by Bett *et al.*, *Proc. Natl. Acad. Sci.*, *USA*, 91:8802-8806 (1994) (see, also International PCT application No. W0 95/00655) using methods well known to one of skill in the art. This vector also is commercially available from Microbix Biosystems and contains an Ad5 genome with the packaging signals at the left end deleted and the E3 region (nucleotides 28133:30818) replaced by a linker with a unique site for the restriction enzyme Pacl. An 11.9 kb BamHI fragment, which contains the right end of the adenovirus genome, is isolated from pBHG10 and cloned into the BamHI site of pBS/SK(+) to create plasmid p11.3 having approximately 14,658 bp. The p11.3 plasmid was then digested with Pacl and Sall to remove the fiber, E4, and inverted terminal repeat (ITR) sequences.

This fragment was replaced with a 3.4 kb fragment containing the ITR segments and the E4 gene which was generated by PCR amplification from pBHG10 using the following oligonucleotide sequences: 5' TGTACACCG GATCCGGCGCACACC3' SEQ ID NO: 104; and 5'CACAACGAGCTC AATTAATTAATTGCCACATCCTC3' SEQ ID NO: 105. These primers incorporated sites for Pacl and BamHI. Cloning this fragment into the Pacl and blunt ended Sall sites of the p11.3 backbone resulted in a substitution of the fused ITRs, E4 region and fiber gene present in pBHG10, by the ITRs and E4 region alone. The resulting

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p11.3 plasmid containing the ITR and E4 regions, designated plasmid pDV43a, was then digested with BamHI. This BamHI fragment was then used to replace a BamHI fragment in pBHG10 thereby creating pDV44 in a pBHG10 backbone.

In an alternative approach to preparing pDV44 with an additional subcloning step to facilitate the incorporation of restriction cloning sites, the following cloning procedure was performed. pDV44 as above was constructed by removing the fiber gene and some of the residual E3 sequences from pBHG10 (Microbix Biosystems; see, also U.S. Patent No. 6,140,087). As above, to simplify manipulations, the 11.9 kb BamHI

fragment including the rightmost part of the Ad5 genome was removed from pBHG10 and inserted into pBS/SK. The resulting plasmid was termed p11.3. The 3.4 kb DNA fragment corresponding to the E4 region and both ITRs of adenovirus type 5 was amplified as described above from pBHG10 using the oligonucleotides listed above and subcloned into

from pBHG10 using the oligonucleotides listed above and subcloned into the vector pCR2.1 (Invitrogen) to create pDV42. This step is the additional cloning step to facilitate the incorporation of a Sall restriction site. pDV42 was then digested with Pacl, which cuts at a unique site (bold type) in one of the PCR primers, and with Sall, which cuts at a unique site in the pCR2.1 polylinker. This fragment was used to replace the corresponding Pacl/Xhol fragment of p11.3 (the pBS polylinker)

pDV43. A plasmid designated pDV44 was constructed by replacing the 11.9 kb BamHI fragment of pBHG10 by the analogous BamHI fragment of pDV43. As generated in the first procedure, pDV44 therefore differs from pBHG10 by the deletion of Ad5 nucleotides 30819:32743 (residual E3 sequences and all but the 3'-most 41 nucleotides of the fiber open reading frame).

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adjacent to the Ad DNA fragment contains a unique Xhol site), creating

In summary, the cloning procedures described above result in the production of a fiber-deleted Ad5 genomic plasmid (pDV44) that was constructed by removing the fiber gene and some of the residual E3 sequences from pBHG10. pDV44 contains a wild-type E4 region, but only the last 41 nucleotides of the fiber ORF (this sequence was retained to avoid affecting expression of the adjacent E4 transcription unit). Plasmids pBHG10 and pDV44 contain unpackageable Ad5 genomes, and must be rescued by cotransfection and subsequent homologous recombination with DNA carrying functional packaging signals. In order to generate vectors marked with a reporter gene, either pDV44 or pBHG10 was cotransfected with pΔE1Bßgal, which contains the left end of the Ad5 genome with an SV40-driven β-galactosidase reporter gene inserted in place of the E1 region.

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In general, and as described below, the method for virus production by recombination of plasmids followed by complementation in cell culture involves the isolation of recombinant viruses by cotransfection of any adenovirus packaging cell system, namely 211A, 211B, 211R, A549, Vero cells, and the like, with plasmids carrying sequences corresponding to viral gene delivery vectors.

20 A selected cell line is plated in dishes and cotransfected with pDV44 and pΔE1Bβ gal using the calcium phosphate method as described by Bett *et al.*, *Proc. Natl. Acad. Sci., USA*, *91*:8802-8806 (1994). Recombination between the overlapping adenovirus sequences in the two plasmids leads to the creation of a full-length viral chromosome where pDV44 and pΔE1Bβ gal recombine to form a recombinant adenovirus vector having multiple deletions. The deletion of E1 and of the fiber gene from the viral chromosome is compensated for by the sequences integrated into the packaging cell genome, and infectious virus particles

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are produced. The plaques thus generated are isolated and stocks of the recombinant virus are produced by standard methods.

Because of the fiber deletion, a pDV44-derived virus is replication-defective, and cells in which it is grown must complement this defect. The 211B cell line (a derivative of 293 cells which expresses the wild-type (wt) AD5 fiber and is equivalent to 211A on deposit with ATCC) was used for rescue and propagation of the virus described here. pDV44 and pΔE1ßgal were cotransfected into 211B cells, and the monolayers were observed for evidence of cytopathic effect (CPE). Briefly, for virus construction, cells were transfected with the indicated plasmids using the Gibco Calcium Phosphate Transfection system according to the manufacturer's instructions and observed daily for evidence of CPE.

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One of a total of 58 transfected dishes showed evidence of spreading cell death at day 15. A crude freeze-thaw lysate was prepared from these cells and the resulting virus (termed Ad5.ßgal.ΔF) was plaque purified twice and then expanded. To prepare purified viral preparations, cells were infected with the indicated Ad and observed for completion of CPE. Briefly, at day zero, 211B cells were plated in DMEM plus 10% fetal calf serum at approximately 1 X 107 cells/150 cm2 flask or equivalent density. At day one, the medium was replaced with one half the original volume of fresh DMEM containing the indicated Ad, in this case Ad5.ßgal.ΔF, at approximately 100 particles/cell. At day two, an equal volume of medium was added to each flask and the cells were observed for CPE. Two to five days after infection, cells were collected and virus isolated by lysis via four rapid freeze-thaw cycles. Virus was then purified by centrifugation on preformed 15-40% CsCl gradients (111,000 x g for three hours at 4° C). The bands were harvested, dialyzed into storage buffer (10 mM Tris-pH 8.1, 0.9% NaCl, and 10% glycerol), aliquoted and stored at - 70°C. Purified Ad5.βgal.ΔF virus

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particles containing human adenovirus Ad5.ßgal.ΔFgenome (described further below) have been deposited with the ATCC on January 15, 1999.

For viral titering, Ad preparations were titered by plaque assay on 211B cells. Cells were plated on polylysine-coated 6 well plates at 1.5 x 10⁶ cells/well. Duplicate dilutions of virus stock were added to the plates in 1 ml/well of complete DMEM. After a five hour incubation at 37°C, virus was removed and the wells overlaid with 2 ml of 0.6% low-melting agarose in Medium 199 (Gibco). An additional 1 ml of overlay was added at five day intervals.

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As a control, the first-generation virus Ad5.ß gal.wt, which is identical to Ad5.ßgal. Δ F except for the fiber deletion, was constructed by cotransfection of pBHG10 and p Δ E1Bßgal. In contrast to the low efficiency of recovery of the fiberless genome (1/58 dishes), all of 9 dishes cotransfected with p Δ E1B β gal and pBHG10 produced virus.

In another embodiment, a delivery plasmid is prepared that does not require the above-described recombination events to prepare a viral vector having a fiber gene deletion. In one embodiment, a single delivery plasmid containing all the adenoviral genome necessary for packaging but lacking the fiber gene is prepared from plasmid pFG140 containing full-length Ad5 that is commercially available from Microbix. The resultant delivery plasmid referred to as pFG140-f is then used with pCLF (ATCC accession number 97737; and described in copending U.S. application Serial No. 09/482,682 (also filed as International PCT application No. PCT/US00/00265 on January 14, 2000)) stably integrated cells as described above to prepare a viral vector lacking fiber. For genetic therapy, the fiber gene can be replaced with a therapeutic gene of interest for preparing a therapeutic delivery adenoviral vector.

Vectors for the delivery of any desired gene and preferably a therapeutic gene are prepared by cloning the gene of interest into the

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multiple cloning sites in the polylinker of commercially available pΔE1sp1B (Microbix Biosystems; see also, U.S. Patent No. 6,140,087), in an analogous manner as performed for preparing pE1Bβ gal as described above. The same cotransfection and recombination procedure is then followed as described herein to obtain viral gene delivery vectors.

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1. Characterization of the Ad5. β gal. Δ F Genome

To confirm that the vector genomes had the proper structures and that the fiber gene was absent from the Ad5.ßgal.ΔF chromosome, the DNA isolated from viral particles was analyzed. Briefly, purified viral DNA was obtained by adding 10 μ l of 10 mg/ml proteinase K, 40 μ l of 0.5 M EDTA and 50 μ l of 10% SDS to 800 μ l of adenovirus-containing culture supernatant. The suspension was then incubated at 55°C for 60 minutes. The solution was then extracted once with 400 μ l of a 24:1 mixture of chloroform:isoamyl alchohol. The aqueous phase was then removed and precipitated with sodium acetate/ethanol. The pellet was washed once with 70% ethanol and lightly dried. The pellet was then suspended in 40 μ l of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Genomic DNA from Ad5.ßgal.wt and Ad5.ßgal.ΔF produced the expected restriction patterns following digestion with either EcoRI or with Ndel. Southern blotting, performed with standard methods, with labeled fiber DNA as a probe demonstrated the presence of fiber sequence in Ad5. Rgal. wt but not in Ad5. Rgal. ΔF DNA. As a positive control, the blot was stripped and reprobed with labeled E4 sequence. Fiber and E4 sequences were detected by using labeled inserts from pCLF and pE4/Hygro, respectively. E4 signal was readily detectable in both genomes at equal intensities. The complete nucleotide sequence of Ad5.ßgal.ΔF is presented in SEQ ID NO: 106 and is contained in the virus particle deposited with ATCC.

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2. Characterization of the Fiberless Adenovirus Ad5.βgal.ΔF

To verify that Ad5.ßgal.ΔF was fiber-defective, 293 cells (which are permissive for growth of E1-deleted Ad vectors but do not express fiber) were infected with Ad5.ßgal.ΔF or with Ad5.ßgal.wt. Twenty-four hours post infection, the cells were stained with polyclonal antibodies directed either against fiber or against the penton base protein. Cells infected with either virus were stained by the anti-penton base antibody, while only cells infected with the Ad5.ßgal.wt control virus reacted with the anti-fiber antibody. This confirms that the fiber-deleted Ad mutant does not direct the synthesis of fiber protein.

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3. Growth of the Fiber-Deleted Ad5.βgal.ΔF Vector in Complementing Cells

Ad5.ßgal.ΔF was found to readily be propagated in 211B cells. As assayed by protein concentration, CsCl-purified stocks of either Ad5.ßgal.ΔF or Ad5.ßgal.wt contained similar numbers of viral particles. The particles appeared to band normally on CsCl gradients. Infectivity of the Ad5.ßgal.ΔF particles was lower than the Ad5.ßgal.wt control, as indicated by an increased particle/PFU ratio. Ad5.ßgal.ΔF was also found to plaque more slowly than the control virus. When plated on 211B cells, Ad5.ßgal.wt plaques appeared within 5-7 days, while plaques of Ad5.ßgal.ΔF continued to appear until as much as 15-18 days post infection. Despite their slower formation, the morphology of Ad5.ßgal.ΔF plaques was essentially normal.

4. Production of Fiberless Ad5.ßgal.ΔF Particles

As Ad5.ßgal. Δ F represents a true fiber null mutation and its stocks are free of helper virus, the fiber mutant phenotype was readily investigated. A single round of growth in cells (such as 293) which do not produce fiber generating a homogeneous preparation of fiberless Ad allowed for the determination of whether such particles would be stable

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and/or infectious. Either Ad5.ßgal.wt or Ad5.ßgal.ΔF was grown in 293 or 211B cells, and the resulting particles purified on CsCl gradients as previously described. Ad5.ßgal.ΔF particles were readily produced in 293 cells at approximately the same level as the control virus and behaved similarly on the gradients, indicating that there was not a gross defect in morphogenesis of fiberless capsids.

Particles of either virus contained similar amounts of penton base regardless of the cell type in which they were grown. This demonstrated that fiber is not required for assembly of the penton base complex into virions. The Ad5.ßgal.ΔF particles produced in 293 cells did not contain fiber protein. 211B-grown Ad5.ßgal.ΔF also contained less fiber than the Ad5.ßgal.wt control virus. The infectivities of the different viral preparations on epithelial cells correlated with the amount of fiber protein present. The fiberless Ad particles were several thousand-fold less infectious than the first-generation vector control on a per-particle basis, while infectivity of 211B-grown Ad5.ßgal.ΔF was only 50-100 fold less than that of Ad5.ßgal.wt. These studies confirmed fiber's crucial role in infection of epithelial cells via CAR binding.

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5. Composition and Structure of the Fiberless Ad5.ßgal.ΔF Particles

The proteins contained in particles of 293-grown Ad5.ßgal. Δ F were compared to those in Ad5.ßgal.wt, to determine whether proteolysis or particle assembly was defective in this fiber null mutant. The overall pattern of proteins in the fiberless particles was observed to be quite similar to that of a first-generation vector, with the exception of reduced intensity of the composite band resulting from proteins IIIa and IV (fiber). The fiberless particles also had a reduced level of protein VII. Although substantial amounts of uncleaved precursors to proteins VI, VII, and VIII were not seen, it is possible that the low-molecular weight bands

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migrating ahead of protein VII represent either aberrantly cleaved viral proteins or their breakdown products.

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Cryo-electron microscopy was used to more closely examine the structure of the 293 grown Ad5.ßgal. AF and of Ad5ßgal.wt. The fiber, having an extended stalk with a knob at the end, was faintly visible in favorable orientations of wild-type Ad5 particles, but not in images of the fiberless particles. Filamentous material likely corresponding to free viral DNA was seen in micrographs of fiberless particles. This material was also present in micrographs of the first-generation control virus, albeit at much lower levels.

Three-dimensional image reconstructions of fiberless and wild-type particles at $\sim\!20$ Å resolution showed similar sizes and overall features, with the exception that fiberless particles lacked density corresponding to the fiber protein. The densities corresponding to other capsid proteins, including penton base and proteins IIIa, VI, and IX, were comparable in 15 the two structures. This confirms that absence of fiber does not prevent assembly of these components into virions. The fiber was truncated in the wild-type structure as only the lower portion of its flexible shaft follows icosahedral symmetry. The RGD protrusions on the fiberless penton base were angled slightly inward relative to those of the wild-type 20 structure. Another difference between the two penton base proteins was that there is a $\sim 30~\text{Å}$ diameter depression in the fiberless penton base around the five-fold axis where the fiber would normally sit. The Ad5 reconstructions confirm that capsid assembly, including addition of penton base to the vertices, is able to proceed in the complete absence of fiber.

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6. Integrin-Dependent Infectivity of Fiberless Ad5.ßgal.ΔF Particles

While attachment via the viral fiber protein is a critical step in the infection of epithelial cells, an alternative pathway for infection of certain hematopoietic cells has been described. In this case, penton base mediates binding to the cells (via ß2 integrins) and internalization (through interaction with av integrins). Particles lacking fiber might therefore be expected to be competent for infection of these cells, even though on a per-particle basis they are several thousand-fold less infectious than normal Ad vectors on epithelial cells.

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To investigate this, THP-1 monocytic cells were infected with Ad5.ßgal.wt or with Ad5.ßgal. \Delta F grown in the absence of fiber. Infection of THP-1 cells was assayed by infecting 2 x 10⁵ cells at the indicated m.o.i. in 0.5 ml of complete RPMI. Forty-eight hours post-infection, the cells were fixed with glutaraldehyde and stained with X-gal, and the percentage of stained cells was determined by light microscopy. The results of the infection assay showed that the fiberless particles were only a few-fold less infectious than first-generation Ad on THP-1 cells. Large differences were seen in plaquing efficiency on epithelial (211B) cells. Infection of THP-1 cells by either Ad5.βgal.ΔF or Ad5. Rgal. wt was not blocked by an excess of soluble recombinant fiber protein, but could be inhibited by the addition of recombinant penton base). These results indicate that the fiberless Ad particles use a fiber-independent pathway to infect these cells. Furthermore, the lack of fiber protein did not prevent Ad5.ßgal∆F from internalizing into the cells and delivering its genome to the nucleus, demonstrating that fiberless particles are properly assembled and are capable of uncoating.

The foregoing results with the recombinant viruses thus produced indicates that they can be used as gene delivery tools in cultured cells

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and *in vivo*. For example, for studies of the effectiveness and relative immunogenicity of multiply-deleted vectors, virus particles are produced by growth in packaging lines and are purified by CsCl gradient centrifugation. Following titering, virus particles are administered to mice via systemic or local injection or by aerosol delivery to lung. The LacZ reporter gene allows the number and type of cells which are successfully transduced to be evaluated. The duration of transgene expression is evaluated in order to determine the long-term effectiveness of treatment with multiply-deleted recombinant adenoviruses relative to the standard technologies which have been used in clinical trials to date. The immune response to the improved vectors described here is determined by assessing parameters such as inflammation, production of cytotoxic T lymphocytes directed against the vector, and the nature and magnitude of the antibody response directed against viral proteins.

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Versions of the vectors which contain therapeutic genes such as CFTR for treatment of cystic fibrosis or tumor suppressor genes for cancer treatment are evaluated in the animal system for safety and efficiency of gene transfer and expression. Following this evaluation, they are used as experimental therapeutic agents in human clinical trials.

B. Retargeting of Adenoviral Gene Delivery Vectors by Producing Viral Particles Containing Different or Altered Fiber Proteins

As the specificity of adenovirus binding to target cells is largely determined by the fiber protein, viral particles that incorporate modified fiber proteins or fiber proteins from different adenoviral serotypes (pseudotyped vectors) have different specificities. Thus, the methods of expression of the native Ad5 fiber protein in adenovirus packaging cells as described above also is applicable to production of different fiber proteins.

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Chimeric fiber proteins can be produced according to known methods (see, e.g., Stevenson et al. (1995) J. Virol., 69:2850-2857). Determinants for fiber receptor binding activity are located in the head domain of the fiber and an isolated head domain is capable of trimerization and binding to cellular receptors. The head domains of adenovirus type 3 (Ad3) and Ad5 were exchanged in order to produce chimeric fiber proteins. Similar constructs for encoding chimeric fiber proteins for use in the methods herein are contemplated. Thus, instead of using the intact Ad5 fiber-encoding construct (prepared above and in U.S. application Serial No. 09/482,682) as a complementing viral vector in adenoviral packaging cells, the constructs described herein are used to transfect cells along with E4 and/or E1-encoding constructs.

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Briefly, full-length Ad5 and Ad3 fiber genes were amplified from purified adenovirus genomic DNA as a template. The Ad5 and Ad3 nucleotide sequences are available with the respective GenBank Accession Numbers M18369 and M12411. Oligonucleotide primers are designed to amplify the entire coding sequence of the full-length fiber genes, starting from the start codon, ATG, and ending with the termination codon TAA. For cloning purposes, the 5' and 3' primers contain the respective restriction sites BamHI and NotI for cloning into pcDNA plasmid. PCR is performed as described above.

The resulting products are then used to construct chimeric fiber constructs by PCR gene overlap extension (Horton *et al.* (1990) *BioTechniques, 8*:525-535). The Ad5 fiber tail and shaft regions (5TS; the nucleotide region encoding amino acid residue positions 1 to 403) are connected to the Ad3 fiber head region (3H; the nucleotide region encoding amino acid residue positions 136 to 319) to form the 5TS3H fiber chimera. Conversely, the Ad3 fiber tail and shaft regions (3TS; the nucleotide region encoding amino acid residues positions 1 to 135) are

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connected to the Ad5 fiber head region (5H; the nucleotide region encoding the amino acid residue positions 404 to 581) to form the 3TS5H fiber chimera. The fusions are made at the conserved TLWT (SEQ ID NO: 46) sequence at the fiber shaft-head junction.

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The resultant chimeric fiber PCR products are then digested with BamHI and Notl for separate directional ligation into a similarly digested pcDNA3.1. The TPL sequence is then subcloned into the BamHI for preparing an expression vector for subsequent transfection into 211 cells or into alternative packaging cell systems. The resultant chimeric fiber construct-containing adenoviral packaging cell lines are then used to complement adenoviral delivery vectors as previously described. Other fiber chimeric constructs are obtained with the various adenovirus serotypes using a similar approach.

In an alternative embodiment, the use of modified proteins including with modified epitopes (see, e.g., Michael et al. (1995) Gene Therapy, 2:660-668 and International PCT application Publication No. WO 95/26412, which describe the construction of a cell-type specific therapeutic viral vector having a new binding specificity incorporated into the virus concurrent with the destruction of the endogenous viral binding specificity). In particular, the authors described the production of an adenoviral vector encoding a gastrin releasing peptide (GRP) at the 3' end of the coding sequence of the Ad5 fiber gene. The resulting fiber-GRP fusion protein was expressed and shown to assemble functional fiber trimers that were correctly transported to the nucleus of HeLa cells following synthesis.

Similar constructs are contemplated for use in the complementing adenoviral packaging cell systems for generating new adenoviral gene delivery vectors that are targetable, replication-deficient and less immunogenic. Heterologous ligands contemplated for use herein to

redirect fiber specificity range from as few as 10 amino acids in size to large globular structures, some of which necessitate the addition of a spacer region so as to reduce or preclude steric hindrance of the heterologous ligand with the fiber or prevent trimerization of the fiber protein. The ligands are inserted at the end or within the linker region. Preferred ligands include those that target specific cell receptors or those that are used for coupling to other moieties such as biotin and avidin.

A preferred spacer includes a short 12 amino acid peptide linker composed of a series of serines and alanine flanked by a proline residue at each end using routine procedures known to those of skill in the art. The skilled artisan will be with the preparation of linkers to accomplish sufficient protein presentation and to alter the binding specificity of the fiber protein without compromising the cellular events that follow viral internalization. Moreover, within the context of this disclosure, preparation of modified fibers having ligands positioned internally within the fiber protein and at the carboxy terminus as described below are contemplated for use with the methods described herein.

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The preparation of a fiber having a heterologous binding ligand is prepared essentially as described in the above-cited paper. Briefly, for the ligand of choice, site-directed mutagenesis is used to insert the coding sequence for a linker into the 3' end of the Ad5 fiber construct in pCLF.

The 3' or antisense or mutagenic oligonucleotide encodes a preferred linker sequence of ProSerAlaSerAlaSerAlaSerAlaProGlySer (SEQ ID NO: 107) followed by a unique restriction site and two stop codons, respectively, to allow the insertion of a coding sequence for a selected heterologous ligand and to ensure proper translation termination. Flanking this linker sequence, the mutagenic oligonucloetide contains sequences that overlap with the vector sequence and allow its incorporation into the construct. Following mutagenesis of the pCLF sequence adding the

linker and stop codon sequences, a nucleotide sequence encoding a preselected ligand is obtained, linkers corresponding to the unique restriction site in the modified construct are attached and then the sequence is cloned into linearized corresponding restriction site.

5 The resultant fiber-ligand construct is then used to transfect 211 or the alternative cell packaging systems previously described to produce complementing viral vector packaging systems.

In a further embodiment, intact fiber genes from different Ad serotypes are expressed by 211 cells or an alternative packaging system.

A gene encoding the fiber protein of interest is first cloned to create a plasmid analogous to pCLF, and stable cell lines producing the fiber protein are generated as described above for Ad5 fiber. The adenovirus vector described which lacks the fiber gene is then propagated in the cell line producing the fiber protein relevant for the purpose at hand. As the only fiber gene present is the one in the packaging cells, the adenoviruses produced contain only the fiber protein of interest and therefore have the binding specificity conferred by the complementing protein. Such viral particles are used in studies such as those described above to determine their properties in experimental animal systems.

20 EXAMPLE 22

Preparation and Use of Adenoviral Packaging Cell Lines Containing Plasmids Containing Alternative TPLs

Plasmids containing tripartite leaders (TPLs) have been constructed. The resulting plasmids that contain different selectable markers, such as neomycin and zeocin, were then used to prepare fiber-complementing stable cell lines for use as for preparing adenoviral vectors.

A. pDV60

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Plasmid pDV60 was constructed by inserting the TPL cassette of SEQ ID NO. 88 into the BamHI site upstream of the Ad5 fiber gene in

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pcDNA3/Fiber, a neomycin selectable plasmid (see, *e.g.*, U.S. application Serial No. 09/482,682 (also filed as International PCT application No. PCT/US00/00265 on January 14, 2000); see also Von Seggern *et al.* (1998) *J. Gen Virol.*, 79: 1461-1468). The nucleotide sequence of pDV60 is listed in SEQ ID NO: 108. Plasmid pDV60 is available from the ATCC under accession number PTA-1144.

B. pDV61

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To construct pDV61, an Asp718/NotI fragment containing the CMV promoter, partial Ad5 TPL, wildtype Ad5 fiber gene, and bovine growth hormone terminator was transferred from pCLF (ATCC accession number 97737; and described in copending U.S. application Serial No. 09/482,682 (also filed as International PCT application No. PCT/US00/00265 on January 14, 2000)), to a zeocin selectable cloning vector referred to as pCDNA3.1/Zeo (+) (commercially available from Invitrogen and for which the sequence is known).

C. pDV67

In an analogous process, pDV67 containing complete TPL was constructed by transferring an Asp 718/Xbal fragment from pDV60 into pcDNA3.1/Zeo(+) backbone. The nucleotide sequence of pDV67 is set forth in SEQ ID NO. 109. Plasmid pDV67 is available from the ATCC under accession number PTA-1145.

D. pDV69

To prepare pDV69 containing a modified fiber protein, the chimeric Ad3/Ad5 fiber gene was amplified from pGEM5TS3H (Stevenson *et al.* (1995) *J. Virol., 69:* 2850-2857) using the primers 5'ATGGGAT CAAGATGAAGCGCGCAAGACCG3' (SEQ ID NO. 110) and 5'CACTATAGCGGCCGCATTCTCAGTCATCTT3' (SEQ ID NO. 111), and cloned to the BamHI and Notl sites of pcDNA3.1/Zeo(+) via new BamHI and Notl sites engineered into the primers to create pDV68. Finally, the

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complete TPL fragment described above was then added to the unique BamHI site of pDV68 to create pDV69. The nucleotide sequence of pDV69 is listed in SEQ ID NO. 112 and the plasmid is available from the ATCC under accession number PTA-1146.

5 E. Preparation of Stable Adenovirus Packaging Cell Lines

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E1-2a S8 cells are derivatives of the A549 lung carcinoma line (ATCC # CCL 185) with chromosomal insertions of the plasmids pGRE5-2.E1 (also referred to as GRE5-E1-SV40-Hygro construct and listed in SEQ ID NO. 47) and pMNeoE2a-3.1 (also referred to as MMTV-E2a-SV40-Neo construct and listed in SEQ ID NO. 48), which provide complementation of the adenoviral E1 and E2a functions, respectively. This line and its derivatives were grown in Richter's modified medium (BioWhitaker) + 10% FCS. E1-2a S8 cells were electroporated as previously described (Von Seggern *et al.* (1998) *J. Gen Virol.*, 79: 1461-1468) with pDV61, pDV67, or with pDV69, and stable lines were selected with zeocin (600 μ g/ml).

The cell line generated with pDV61 is designated 601. The cell line generated with pDV67 is designated 633 while that generated with pDV69 is designated 644. Candidate clones were evaluated by immunofluorescent staining with a polyclonal antibody raised against the Ad2 fiber. Lines expressing the highest level of fiber protein were further characterized.

For the S8 cell complementing cell lines, to induce E1 expression, 0.3 μ M of dexamethasone was added to cell cultures 16-24 hours prior to challenge with virus for optimal growth kinetics. For preparing viral plaques, 5 X 10⁵ cells/well in 6 well plates are prepared and pre-induced with the same concentration of dexamethasone the day prior to infection with 0.5 μ M included at a final concentration in the agar overlay after infection.

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F. Cell Lines for Complementation of E1/E2a Vectors

The Adenovirus 5 genome was digested with Scal enzyme, separated on an agarose gel, and the 6,095 bp fragment containing the left end of the virus genome was isolated. The complete Adenovirus 5 genome is registered as Genbank accession #M73260 (or see SEQ ID NO. 1), incorporated herein by reference, and the virus is available from the American Type Culture Collection, Manassas, Virginia, U.S.A., under accession number VR-5. The Scal 6,095 bp fragment was digested further with Clal at bp 917 and Bglll at bp 3,328. The resulting 2,411 bp Clal to Bglll fragment was purified from an agarose gel and ligated into the superlinker shuttle plasmid pSE280 (Invitrogen, San Diego, CA), which was digested with Clal and Bglll, to form pSE280-E.

Polymerase chain reaction (PCR) was performed to synthesize DNA encoding an Xhol and Sall restriction site contiguous with Adenovirus 5 DNA bp 552 through 924. The primers which were employed were as follows:

5' end, Ad5 bp 552-585:

5'-GTCACTCGAGGACTCGGTC-GACTGAAAATGAGACATATTATCTGCC ACGGACC-3' (SEQ ID NO. 113)

20 3' end, Ad5 bp 922-891:

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5'-CGAGATCGATCACCTCCGGTACAAGGTTTGGCATAG-3' (SEQ ID NO. 114)

This amplified DNA fragment (also referred to herein as Fragment A) was digested with Xhol and Clal, which cleaves at the native Clal site (bp 917), and ligated to the Xhol and Clal sites of pSE280-E, thus reconstituting the 5' end of the E1 region beginning 8 bp upstream of the ATG codon. PCR amplification then was performed to amplify Ad 5 DNA from bp 3,323 through 4,090 contiguous with an EcoRl restriction site. The primers employed were as follows:

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5' end, Ad5 bp 3323-3360:

5'-CATGAAGATCTGGAAGGTGCTGAGGTACGATGAGACC-3' (SEQ ID NO. 115); and

3' end, Ad5 bp 4090-4060:

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5'-GCGACTTAAGCAGTCAGCTG-AGACAGCAAGACACTTGCTTGATCCA AATCC-3' (SEQ ID NO. 116).

This amplified DNA fragment (also referred to herein as Fragment B) was digested with BgIII, thereby cutting at the Adenovirus 5 BgIII site (bp 3,382) and EcoRI, and ligated to the BgIII and EcoRI sites of pSE280-AE to reconstruct the complete E1a and E1b region from Adenovirus 5 bp 552 through 4,090. The resulting plasmid is designated pSE280-E1.

A construct containing the intact E1a/b region under the control of the synthetic promoter GRE5 was prepared as follows. The intact E1a/b region was excised from pSE280-E1, which was modified previously to contain a BamHI site 3' to the E1 gene, by digesting with XhoI and BamHI. The XhoI to BamHI fragment containing the E1a/b fragment was cloned into the unique XhoI and BamHI sites of pGRE5-2/EBV (U.S. Biochemicals, Cleveland, Ohio) to form pGRE5-E1).

Bacterial transformants containing the final construct were identified. Plasmid DNA was prepared and purified by banding in CsTFA prior to use for transfection of cells.

G. Construction of plasmid including Adenovirus 5 E2A sequence

The Adenovirus 5 genome was digested with BamHI and Spel, which cut at bp 21,562 and 27,080, respectively. Fragments were separated on an agarose gel and the 5,518 bp BamHI to Spel fragment was isolated. The 5,518 bp BamHI to Spel fragment was digested further with Smal, which cuts at bp 23,912. The resulting 2,350 bp BamHI to

Smal fragment was purified from an agarose gel, and ligated into the superlinker shuttle plasmid pSE280, and digested with BamHI and Smal to form pSE280-E2 BamHI-Smal.

PCR then was performed to amplify Adenovirus 5 DNA from the Smal site at bp 23,912 through 24,730 contiguous with Nhel and EcoRI restriction sites. The primers which were employed were as follows: 5' end, Ad5 bp 24,732-24,708: 5'-CACGAATTCGTCAGCGCTTCTCGTCGCGTCCAAGACCC-3' (SEQ ID NO. 117);

10 3' end, Ad5 bp 23,912-23,934:

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5'-CACCCGGGGAGGCGGCGACGGGACGGG-3' (SEQ ID NO. 118)

This amplified DNA fragment was digested with Smal and EcoRI, and ligated to the Smal and EcoRI sites of pSE280-E2 Bam-Sma to reconstruct the complete E2a region from Ad5 bp 24,730 through

15 21,562. The resulting construct is pSE280-E2a.

In order to convert the BamHI site at the 3' end of E2a to a Sall site, the E2a region was excised from pSE280-E2a by cutting with BamHI and Nhel, and recloned into the unique BamHI and Nhel sites of pSE280. Subsequently, the E2a region was excised from this construction with Nhel and Sall in order to clone into the Nhel and Sall sites of the pMAMneo (Clonetech, Palo Alto, CA) multiple cloning site in a 5' to 3' orientation, respectively. The resulting construct is pMAMneo E2a.

Bacterial transformants containing the final pMAMneo-E2a were identified. Plasmid DNA was prepared and purified by banding in CsTFA.

25 Circular plasmid DNA was linearized at the XmnI site within the ampicillin resistance gene of pMAMneo-E2a, and further purified by the phenol/chloroform extraction and ethanol precipitation prior to use for transfection of cells.

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H. Transfection and selection of cells

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In general, this process involved the sequential introduction, by calcium phosphate precipitation, or other means of DNA delivery, of two plasmid constructions each with a different viral gene, into a single tissue culture cell. The cells were transfected with a first construct and selected for expression of the associated drug resistance gene to establish stable integrants. Individual cell clones were established and assayed for function of the introduced viral gene. Appropriate candidate clones then were transfected with a second construct including a second viral gene and a second selectable marker. Transfected cells then were selected to establish stable integrants of the second construct, and cell clones were established. Cell clones were assayed for functional expression of both viral genes.

A549 (ATCC Accession No. CCL-185) were used for transfection.

15 Appropriate selection conditions were established for G418 and hygromycin B by standard kill curve determination.

Transfection of A549 cells with plasmids including E1 and E2a regions.

pMAMNeo-E2a was linearized with XmnI with the Amp^R gene, introduced into cells by transfection, and cells were selected for stable integration of this plasmid by G418 selection until drug resistant colonies arose. The clones were isolated and screened for E2a expression by staining for E2a protein with a polyclonal antiserum, and visualizing by immunofluorescence. E2a function was screened by complementation of the temperature-sensitive mutant Ad5ts125 virus which contains a temperature-sensitive mutation in the E2a gene. (Van Der Vliet, et al., J. Virology, Vol. 15, pgs. 348-354 (1975)). Positive clones expressing the E2a gene were identified and used for transfection with the 7 kb EcoRV to XmnI fragment from pGRE5-E1, which contains the GRE5 promoted

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E1a/b region plus the hygromycin^R gene. Cells were selected for hygromycin resistance and assayed for E1a/b expression by staining with a monoclonal antibody for the E1 protein (Oncogene Sciences, Uniondale, N.Y.). E1 function was assayed by ability to complement an E1-deleted vector. At this point, expression and function of E2a was verified as described above, thus establishing the expression of E1a/b and E2a in the positive cell clones.

A transfected A549 (A549 (ATCC Accession No. CCL-185);) cell line showed good E1a/b and E2a expression and was selected for further characterization. It was designated the S8 cell line.

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I. Preparation of Adenoviral Vectors Containing Ad5.βgal.ΔF Genome in S8 Fiber-Complementing Cell Lines

To prepare adenoviral vectors containing Ad5.βgal.ΔF (Ad5.βgal.ΔF has been was deposited the ATCC under accession number VR2636) in 15 S8 cells containing alternative forms of TPL for enhancing the expression of fiber proteins, the protocol as described in Example 21 for preparing Ad5.βgal.ΔF in 211B cells was followed with the exception of pretreatment with 0.3 μ M dexamethasone for 24 hours as described above. Thus, viral particles with the wildtype Ad5 fiber protein on their surface 20 and containing the fiberless Ad5.βgal.ΔF genome were produced in 633 cells. Particles produced in 644 cells also contained the fiberless Ad5.βgal.ΔF genome, but had the chimeric 5T3H fiber protein, with the Ad3 fiber knob, on their surface. These viral preparations can be used to target delivery of the Ad5. β gal. Δ F, Ad5.GFP. Δ F, or other similarly constructed fiberless genome with either wild-type or modified fibers. 25

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EXAMPLE 23

Enhanced infectivity of dendritic cells by pseudotyped adenoviral particles

Bone marrow-derived dendritic cells were generated by culture of bone marrow cells from female Balb/C mice with GM-CSF and IL-4 (Inaba et al. (1998) Isolation of dendritic cells in Current Protocols in Immunology, John Wiley & Sons, Inc. Philadelphia, 3.7.1-3.7.15). To confirm that the cultured cells expressed surface markers characteristic of dendritic cells, the cells were stained with fluorescently-conjugated antibodies directed against CD11c, CD80, and CD86 and analyzed by fluorescence-activated cell sorting (FACS) analysis. Antibodies against the dendritic cell markers CD11c, CD80 and CD86 are commercially available, such as from eBioscience.

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The primary dendritic cell cultures were infected with 100,000 viral particles/cell of Ad5.GFP.ΔF pseudotyped with either Ad5, Ad16, Ad19p, Ad30, Ad35 or Ad37 fiber. The percent of cells positive for virus-induced GFP expression was determined by FACS analysis 48 hours after infection. All infections were performed in triplicate, and the mean ± standard deviation was determined.

In agreement with previous experiments, Ad5.GFP.ΔF pseudotyped with Ad5 fiber infected dendritic cells poorly with approximately 10% of cells positive for GFP expression, which is likely due to the lack of CAR expression on dendritic cells. In contrast, viruses carrying the Ad16, Ad19p, Ad30, Ad35 or Ad37 fiber proteins demonstrated enhanced infectivity of dendritic cells (approximately 49%, 46%, 37%, 26% and 50% of cells were GFP-positive), indicating that the fiber receptors for these serotypes are expressed on dendritic cells.

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EXAMPLE 24

Subgroup D adenoviruses demonstrate selective infectivity

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Sequence and phylogenetic analysis of adenovirus fiber DNA and amino acid sequence suggests that subgroup B and subgroup D viruses bind different cellular receptors (Havenga *et al.* (2002) *J. Virol.* 76:4612-4620). In addition, while subgroup B viruses (such as Ad16, Ad35 and Ad50), are capable of infecting a wide variety of cancer cell lines and primary cells, including endothelial cells, smooth muscle cells, synoviocytes, fibroblasts, amniocytes, dendritic cells, bone marrow stroma cells, chondrocytes, myoblasts, melanocytes, follicle dermal papilla cells and hematopoietic stem cells (Havenga *et al.* (2002) *J. Virol.* 76:4612-4620), subgroup D viruses have a more selective tropism.

To determine whether select subgroup B (Ad3, Ad16 and Ad35) and subgroup D (Ad19p, Ad30 and Ad37) adenoviruses exhibit the same cellular tropism, a panel of cancer cell lines were tested for their capacity to support Ad gene delivery. The cell lines used were PC-3 cells, HepG2 cells, LNCaP cells and DU 145 cells. These cell lines are available from the ATCC under accession numbers CRL-1435, HB-8065, CRL-10995 and HTB-81, respectively.

Each cell line was infected with either 1000, 5000 or 10,000 particles per cell of Ad5.GFP.ΔF pseudotyped with Ad5 (subgroup C), Ad3, Ad16, Ad19p, Ad30, Ad35 or Ad37 fiber. After 48 hours, virus-directed GFP expression was determined by FACS analysis. For PC-3 cells infected with 1000 particles per cell, little to no GFP expression was detected in cells infected with viruses pseudotyped with subgroup D fibers Ad19p, Ad30 and Ad37. In contrast, GFP-expression was detected in approximately 40% of PC-3 cells infected with Ad16 and Ad35 fiber containing viruses. A similar pattern of GFP expression was found with cells infected at higher multiplicities of infection (MOIs). Approximately

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80% of PC-3 cells infected with 5000 particles per cell of adenoviruses pseudotyped with Ad16 or Ad35 fiber were GFP positive, whereas only 2% of PC-3 cells were GFP positive when infected with Ad19p or Ad30 fiber pseudotyped viruses.

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Similarly, in HepG2 cells, approximately 80% of cells were GFP-positive when infected with 5000 particles per cell of Ad16 or Ad35 fiber pseudotyped viruses, but less than 25% were GFP-positive when infected with either Ad19p or Ad30 fiber pseudotyped viruses. In addition, less than 10% of LNCaP cells were GFP-positive when infected with either 5000 or 10,000 particles per cell of Ad19p, Ad30 or Ad37 fiber containing adenoviruses, whereas Ad16 and Ad35 fiber directed GFP expression in approximately 65% of LNCaP cells. A similar pattern of infection was found in DU 145 cells. These results further demonstrate that subgroup B adenoviruses have a wider cellular tropism than subgroup D viruses and provides additional evidence that subgroup B and subgroup D adenoviruses use different receptors for cell binding and infection.

EXAMPLE 25

Immunization with adenovirus particles pseudotyped with Ad37 fiber results in T-cell stimulation

The following experiment was performed to determine whether immunization of mice with adenoviral particles pseudotyped with fiber protein from subgroup D adenovirus leads to stimulation of CD8+ T cells. Mice (eight in each experimental group, four in the vehicle (control) group) were immunized by subcutaneous injection with 1 x 10¹⁰ particles of either Ad5.GFP.WT (Ad5 particles pseudotyped with Ad5 fiber) or Ad5.GFP.F37 (Ad5 particles pseudotyped with Ad37 fiber). Four weeks following innoculation, spleens were harvested to quantitate stimulation of T cells by determining the number of IFN-y-positive CD8+ T cells.

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To determine the percentage of activated CD8 + T cells in immunized mice, spleens were isolated and mechanically disrupted. Following lysis of red blood cells, 1 x 10⁶ splenocytes were cultured for three hours in RPMI with 10% fetal calf serum and Golgiplug (BD Biosciences), in the presence or absence of 0.1 μg/ml EGFP epitope peptide HYLSTQSAL or the irrelevant OVA peptide (SIINFEKL) as a control. Cells were then stained with an APC-conjugated anti-CD8 antibody (eBioscience), fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) and stained with a PE-conjugated antibody against IFN-γ. The cells were analyzed by fluorescence activated cell sorting (FACS) and the percentage of CD8 + cells positive for IFN-γ was determined ((number of CD8 + IFN-γ + cells divided by the total number of CD8 + T cells) x 100).

Immunization with adenovirus particles pseudotyped with either Ad5 fiber or Ad37 fiber led to stimulation of CD8+ T cells, as indicated by production of IFN-y in these cells. These results indicate adenovirus particles with Ad37 fiber are excellent vaccine candidates due to their ability to stimulate CD8+ T cells while avoiding transduction of liver cells.

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Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An adenovirus particle, comprising a heterologous fiber or a portion thereof, whereby binding of the viral particle to dendritic cells is increased compared to a particle that expresses its native fiber, wherein:

the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber includes fiber from a subgroup D adenovirus for binding to dendritic cells, wherein the subgroup D adenovirus is selected from the group consisting of adenovirus serotype 8, 9, 10, 13, 15, 17, 19a, 19p, 20, 22-30, 32, 33, 36, 38, 39 and 42-49.

2. An adenovirus particle, comprising a heterologous fiber or a portion thereof, whereby binding of the viral particle to heparin sulfate proteoglycans (HSP) is reduced or eliminated compared to a particle that expresses its native fiber, wherein:

the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber comprises fiber from Ad19p or Ad30, whereby HSP interaction is reduced.

3. An adenovirus particle, comprising a heterologous fiber or a portion thereof, whereby binding of the viral particle to dendritic cells is increased compared to a particle that expresses its native fiber, wherein:

the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber comprises fiber from a subgroup B adenovirus for binding the virus to dendritic cells, wherein the subgroup B adenovirus is selected from the group consisting of adenovirus serotype 7, 11, 14, 21, 34 or 50.

4. A particle of any of claims 1-3, wherein:

the fiber is chimeric and comprises an N-terminal portion from a fiber of a subgroup C adenovirus; and

the N-terminal portion is sufficient to increase incorporation into the particle compared to in its absence.

- 5. The particle of claim 1 or 2, wherein the fiber is a chimeric fiber that includes a sufficient portion of a subgroup D adenovirus fiber to target dendritic cells.
- 6. The particle of any of claims 1-5, wherein the subgroup C virus is selected from the group consisting of adenovirus serotype 1, 2, 5, and 6.
- 7. The particle of any of claims 1-6, wherein the fiber is further modified to reduce any interaction with CAR.
- 8. The particle of any of claims 1 and 3-7, wherein the fiber is modified to reduce any interaction with heparin sulfate proteoglycans (HSP).
- 9. The particle of any of claims 1-8, wherein the capsid includes further modifications that alter interaction with a_v integrin.
- 10. The particle of any of claims 1 or 4-9, wherein the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber is from Ad19p.

- 11. The particle of claim 2 or claim 10, wherein the Ad19p fiber comprises at least a sufficient number of amino acids set forth as SEQ ID NO. 34 to target the particle to dendritic cells.
- 12. The particle of claim 11, wherein the Ad19p fiber comprises at least a sufficient number of amino acids set forth as SEQ ID NO. 34 to target the particle to dendritic cells, but exhibits reduced binding to HSP compared to a subgroup C fiber.

- 13. The particle of any of claims 10-12, wherein the fiber is chimeric and includes a portion of a subgroup C adenovirus.
- 14. The particle of any of claims 1 or 4-9, wherein the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber is from Ad30.

- 15. The particle of claim 14, wherein the Ad30 fiber comprises at least a sufficient number of amino acids set forth as SEQ ID NO. 36 to target the particle to dendritic cells.
- 16. The particle of claim 14, wherein the Ad30 fiber comprises at least a sufficient number of amino acids set forth as SEQ ID NO. 36 to target the particle to dendritic cells, but exhibits reduced binding to HSP compared to a subgroup C fiber.
- 17. The particle of any of claims 14-16, wherein the fiber is chimeric and includes a portion of a subgroup C adenovirus.
- 18. An adenovirus particle of any of claims 8-17, comprising a mutation in a CAR-binding region of the capsid to decrease CAR binding.
- 19. An adenovirus particle of any of claims 1-18, comprising a mutation in the α_v integrin-binding region of the capsid, whereby binding to the integrin is eliminated or reduced.
- 20. The adenovirus particle of claim 1, 2, 4-13, 18 or 19, wherein the Ad19p fiber is modified by replacing the N-terminal 15, 16 or 17 amino acids with the 15, 16 or 17 amino acids of an Ad2 or Ad5 fiber.
- 21. The adenovirus particle of claim 14-17, wherein the Ad30 fiber is modified by replacing the N-terminal 15, 16 or 17 amino acids with the 15, 16 or 17 amino acids of an Ad2 or Ad5 fiber.
- 22. The adenovirus particle of claim 7 or claim 18, wherein the CAR-binding region of the capsid that is modified is on a fiber knob.

- 23. The adenovirus particle of claim 22, wherein the fiber protein further comprises one or more further modifications that reduce or eliminate interaction of the resulting fiber with HSP.
- 24. The adenovirus particle of claim 23, wherein the capsid further comprises a ligand, whereby the particle binds to a receptor for the ligand.
- 25. The adenovirus particle of claim 24, wherein the ligand is included in the knob region of the fiber.
- 26. The adenovirus particle of claim 24, wherein the ligand is inserted into the fiber or it replaces a portion of the fiber.
- 27. A particle of any of claims 1-26, further comprising a heterologous nucleic acid in the genome thereof, wherein the heterologous nucleic acid encodes an antigen or a product that alters dendritic cell activity.
- 28. The particle of claim 27, wherein the antigen is a tumor antigen or an antigen from a pathogen.
- 29. A composition formulated for administration to a subject comprising a particle of any of claims 1-28.
- 30. A composition of claim 29 formulated for intramuscular or IV or parenteral administration.
 - 31. A composition of claim 29 or claim 30 that is a vaccine.
- 32. An immunotherapeutic method, comprising administering a composition of any of claims 29-31 to a subject.
- 33. A method of delivering viral particles to dendritic cells, comprising:

contacting a composition with cells that comprise dendritic cells, whereby viral particles bind to dendritic cells, wherein the composition contains a viral particle of any of claims 1 and 3-28 or an adenovirus particle that comprises a fiber from Ad37 for targeting the

particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

infusing the composition into a subject.

- 34. The method of claim 33, wherein the cells are removed from the subject prior to contacting.
- 35. The method of claim 33, wherein the cells comprise immune cells.
- 36. The method of claim 33, wherein the cells are bone marrow cells.
- 37. A nucleic acid molecule encoding a viral particle of any of claims 1-28.
- 38. The nucleic acid molecule of claim 37 that comprises an adenovirus vector.
- 39. The nucleic acid molecule of claim 37 or claim 38 that comprises heterologous nucleic acid.
 - 40. A cell, comprising the nucleic acid of any of claims 37-39.
 - 41. The cell of claim 40 that is a dendritic cell.
- 42. A method of treatment, comprising administering a cell to a subject who has an immune cell disorder, cancer or an infection, wherein the cell is a cell of claim 41 or a dendritic cell containing an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.
- 43. The method of claim 32 or 42, wherein the subject is infected with a pathogen, has a tumor, an inflammatory disorder, allergies, asthma or an autoimmune disease.
- 44. A method of targeting an adenovirus particle to dendritic cells, comprising replacing all or a portion of the native fiber of the adenovirus with an adenovirus subgroup D fiber.

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45. The method of claim 44, wherein:

the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the subgroup D adenovirus is selected from the group consisting of adenovirus serotype 8, 9, 10, 13, 15, 17, 19a, 19p, 20, 22-30, 32, 33, 36, 37, 38, 39 and 42-49.

- 46. A method of targeting an adenovirus particle to dendritic cells, comprising replacing all or a portion of the native fiber of the adenovirus with an adenovirus subgroup B fiber.
- 47. The method of claim 46, wherein:
 the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the subgroup B adenovirus is selected from the group consisting of adenovirus serotype 3, 7, 11, 14, 16, 21, 34, 35, or 50.

- 48. The method of claim 45 or claim 47, wherein the subgroup C virus is selected from the group consisting of adenovirus serotypes 1, 2, 5, and 6.
- 49. The method of claim 45 or claim 47, wherein the fiber is further modified to reduce any interaction with CAR.
- 50. The method of claim 49, wherein the fiber is further modified to reduce any interaction with heparin sulfate proteoglycans (HSP).
- 51. The method of claim 50, wherein the capsid includes further modifications that alter interaction with α_v integrin.
- 52. Use of an adenovirus particle for treatment of a disorder or disease, wherein the particle is a particle of any of claims 1-28 or is an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.

- 53. The use of claim 52, wherein the disease or disorder is an immune cell disorder, cancer or an infection.
- 54. Use of an adenovirus particle for preparation of a medicament for the treatment of an immune cell disorder, cancer or an infection, wherein the particle is a particle of any of claims 1-28 or is an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.
- 55. Use of a cell for treatment of disease or disorder selected from an immune cell disorder, cancer and an infection, wherein the cell is a cell of claim 41 or a dendritic cell containing an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.
- 56. The use of claim 55, wherein the disorder is a tumor, an inflammatory disorder, allergies, asthma or an autoimmune disease.
- 57. Use of a cell for the preparation of a medicament for the treatment of a disease or disorder selected from among an immune cell disorder, cancer and an infection, wherein the cell is a cell of claim 41 or a dendritic cell containing an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.
- 58. The use of claim 57, wherein the disease or disorder is a tumor, an inflammatory disorder, allergies, asthma or an autoimmune disease.

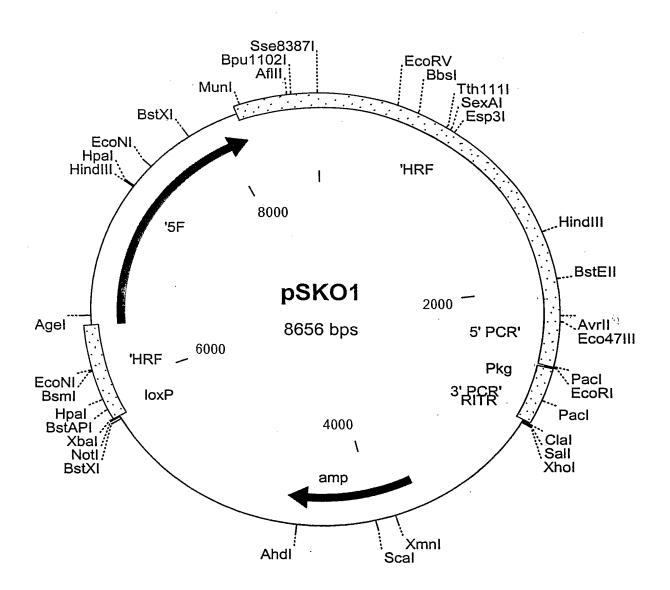


FIG. 1

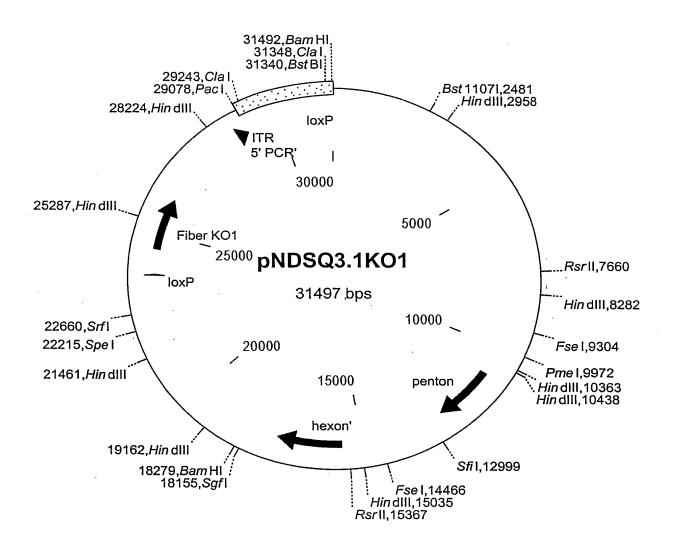


FIG. 2

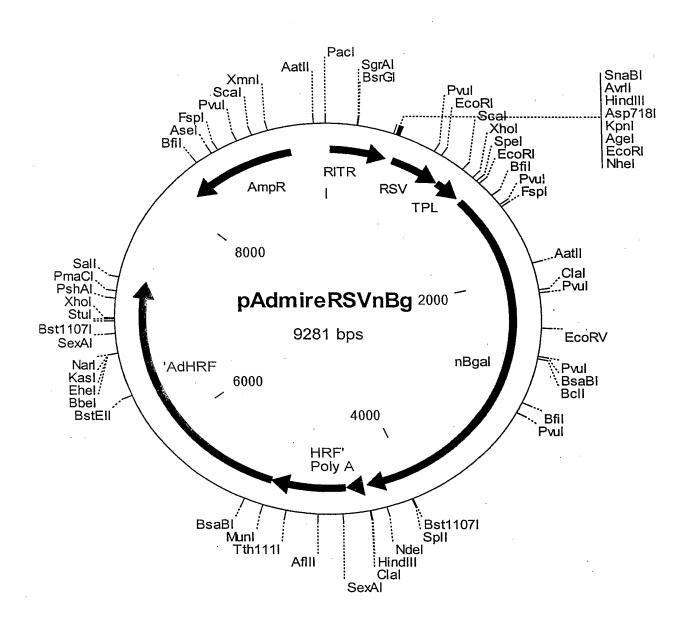


FIG. 3A

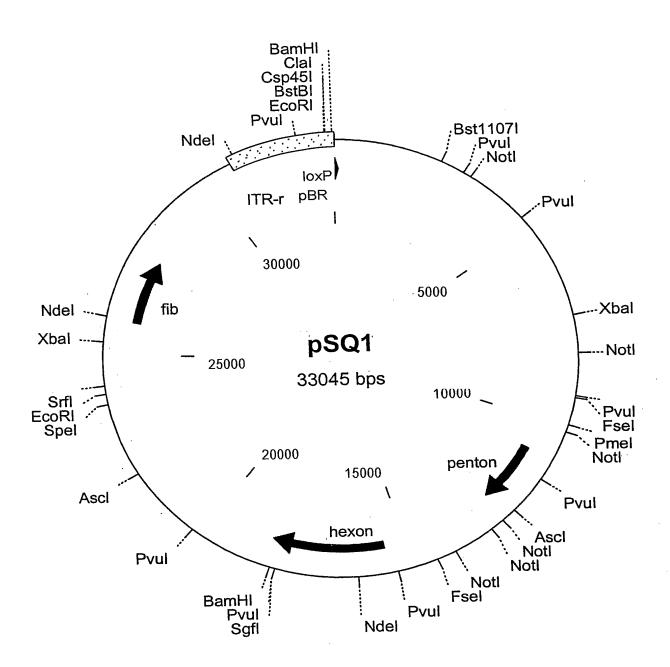


FIG. 3B

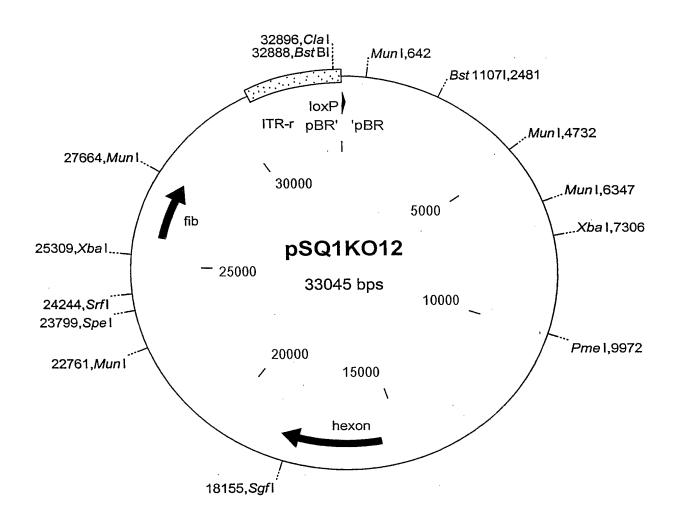


FIG. 3C

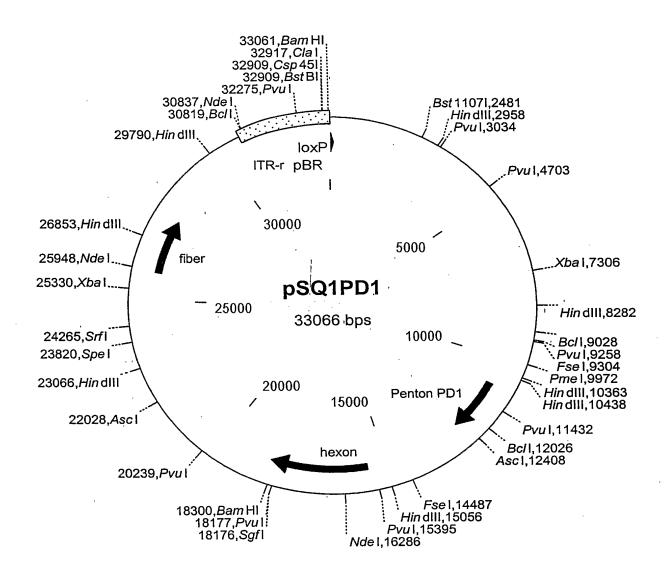


FIG. 4

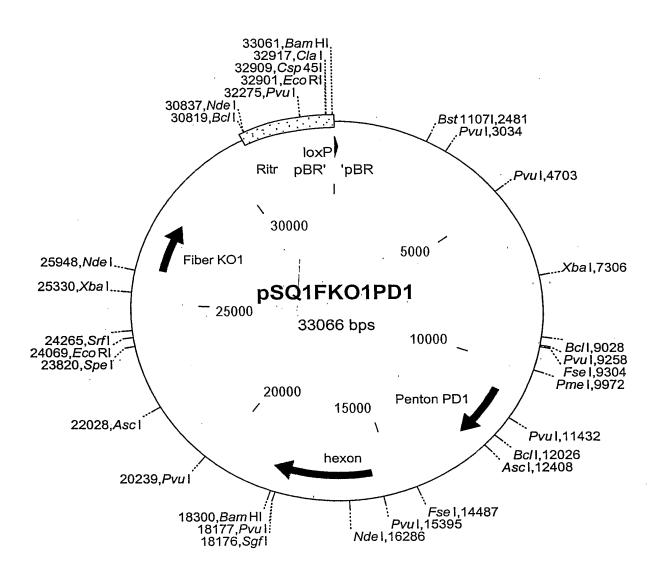


FIG. 5A

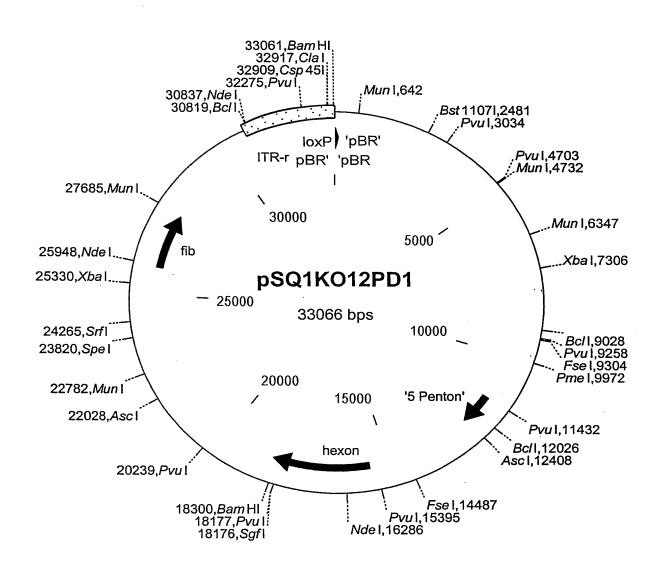


FIG. 5B

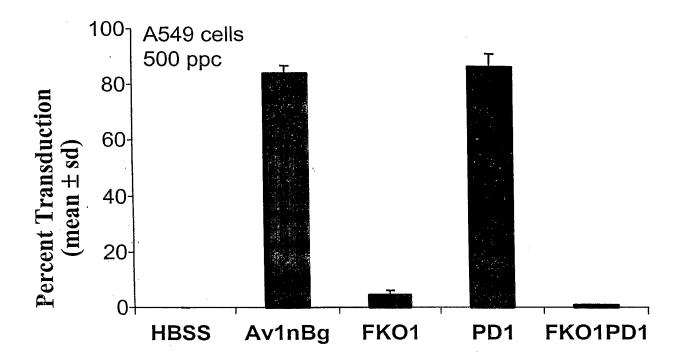
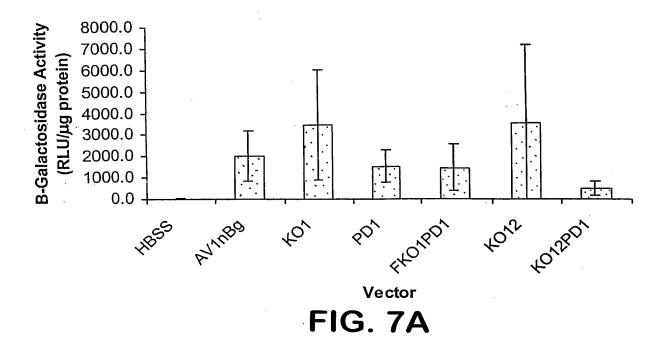
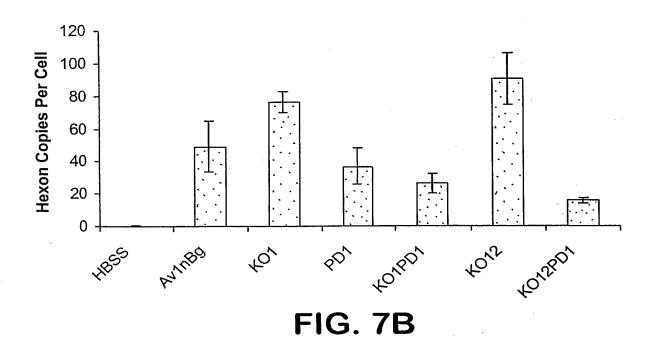


FIG. 6





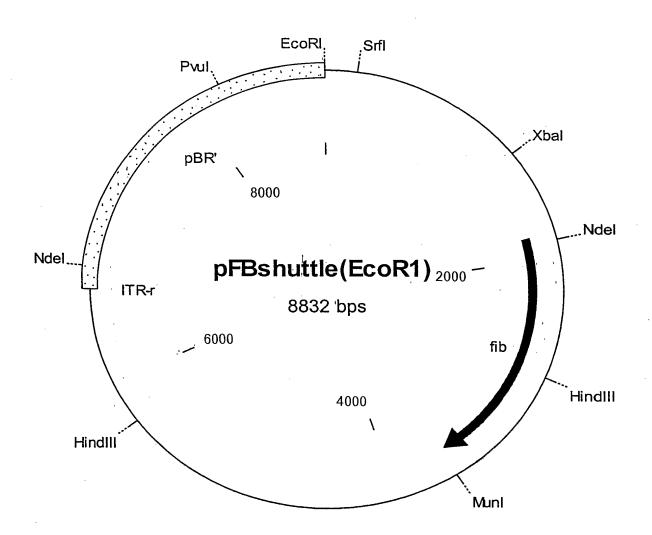


FIG. 8

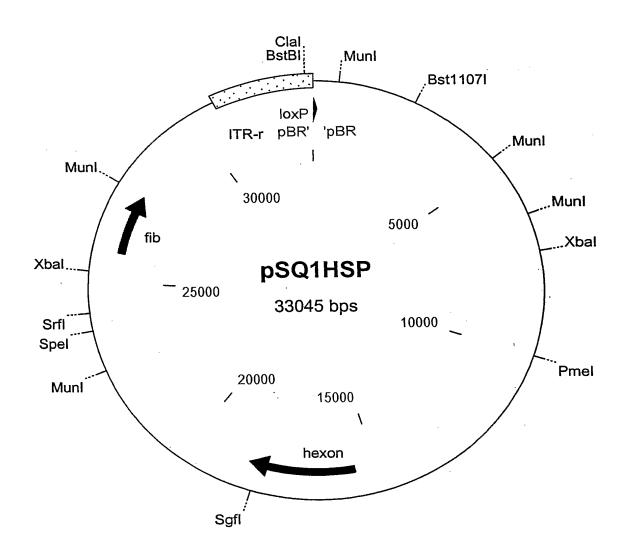


FIG. 9

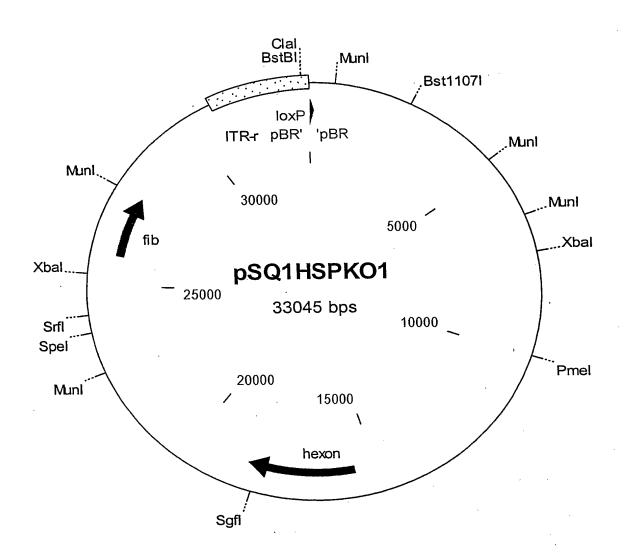


FIG. 10

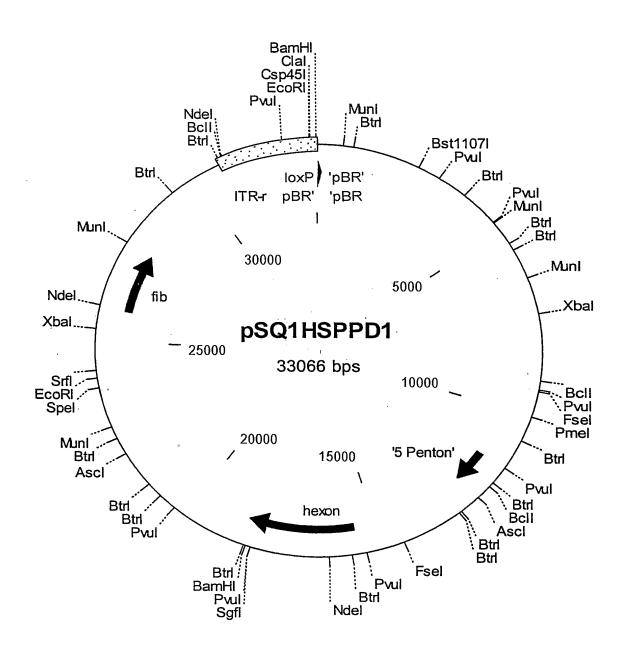


FIG. 11

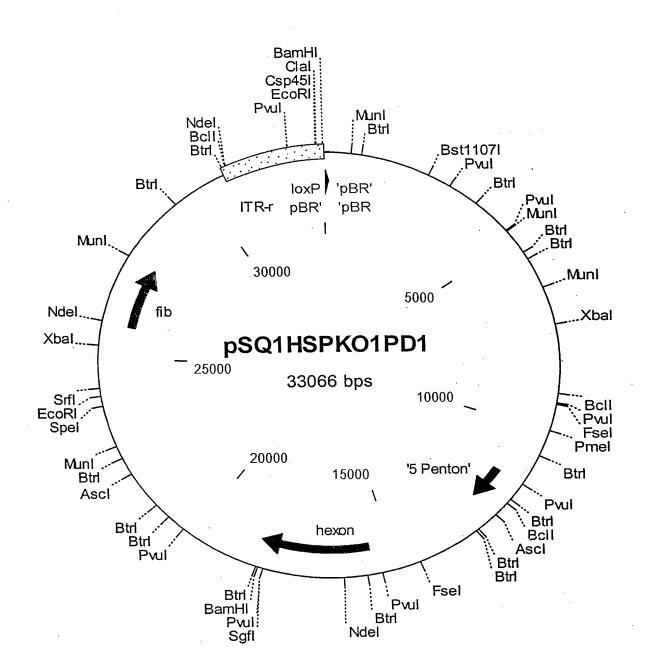


FIG. 12

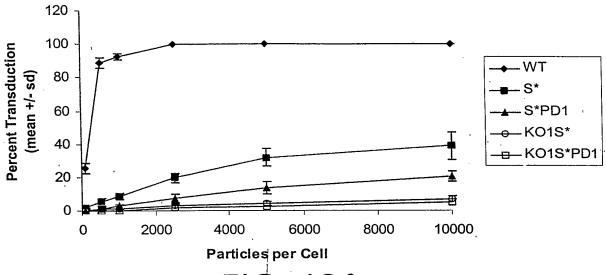


FIG. 13A

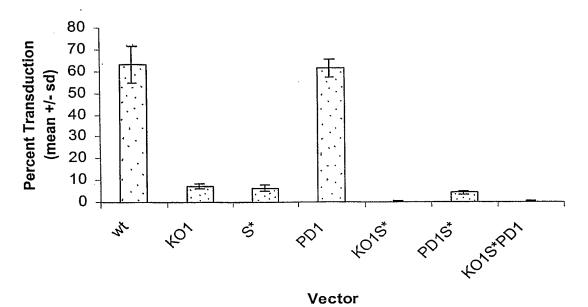


FIG. 13B

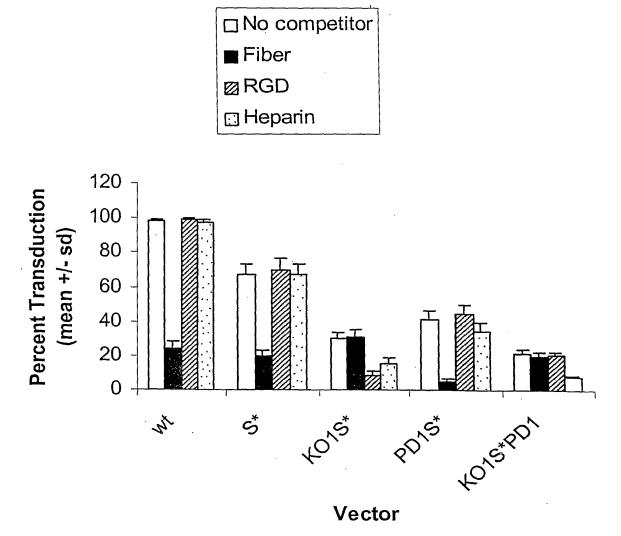


FIG. 13C

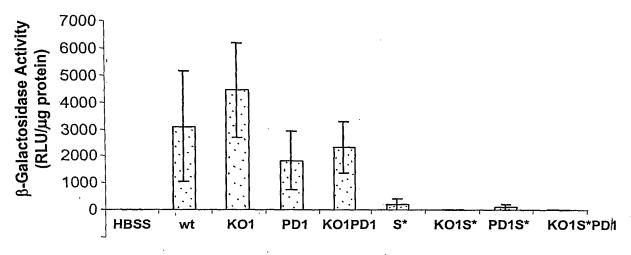


FIG. 14A

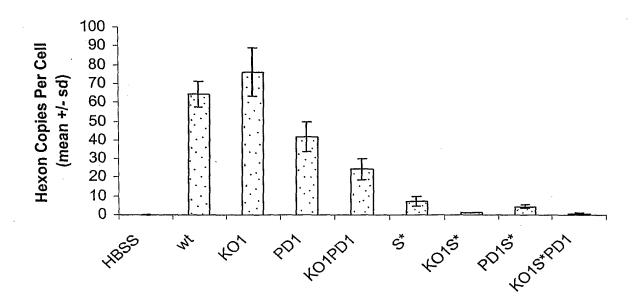


FIG. 14B

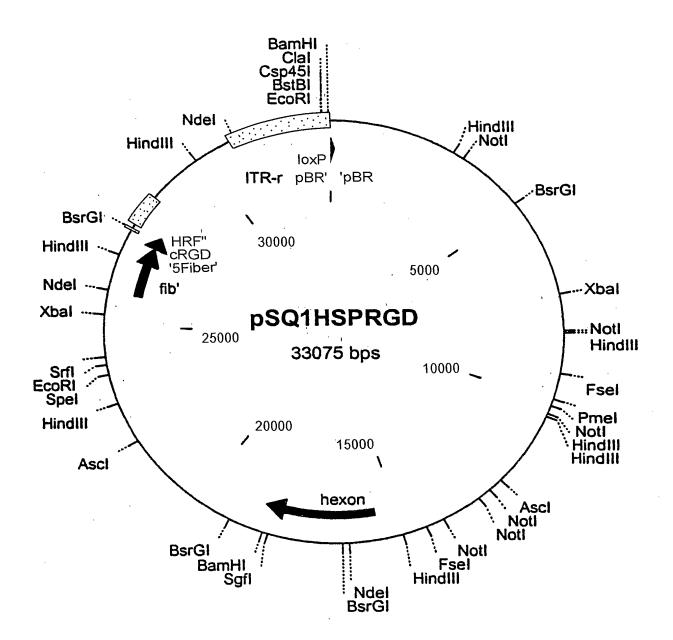


FIG. 15A

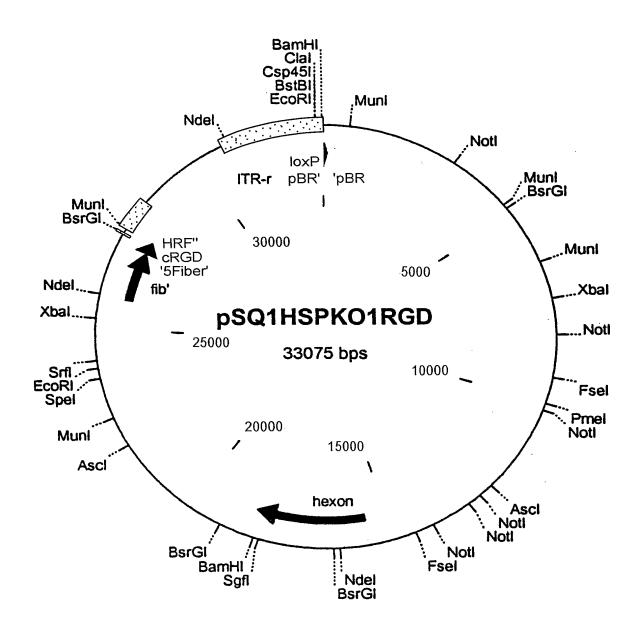


FIG. 15B

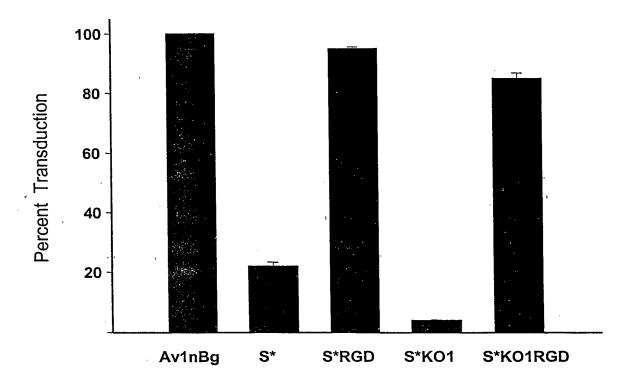


FIG. 16

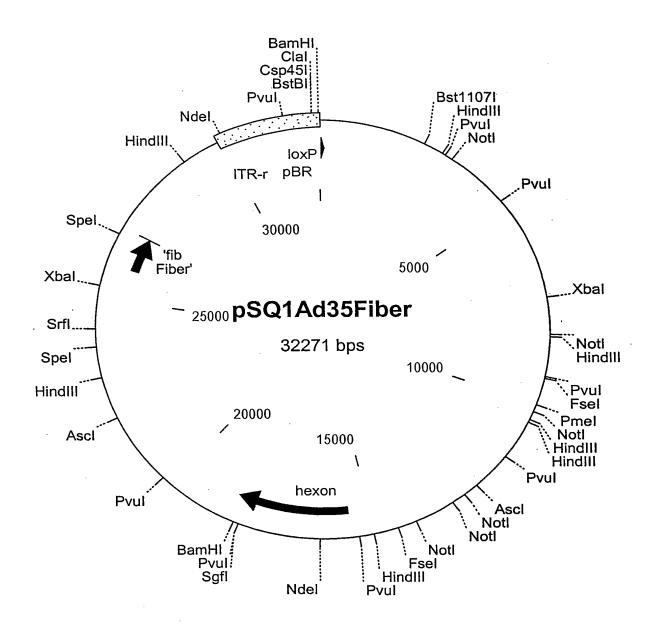


FIG. 17A

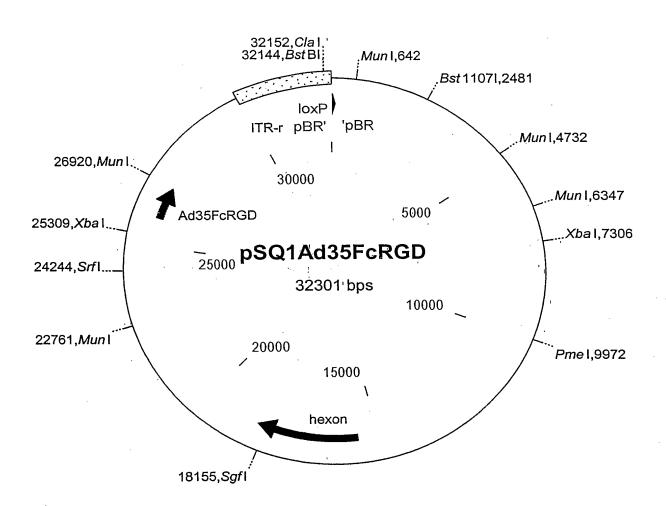


FIG. 17B

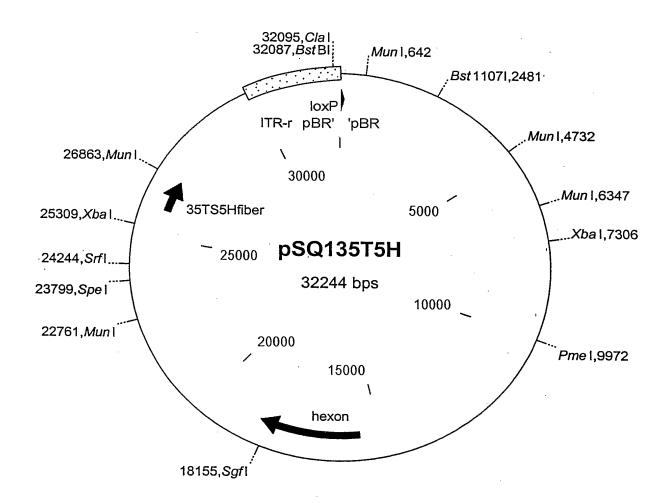


FIG. 18A

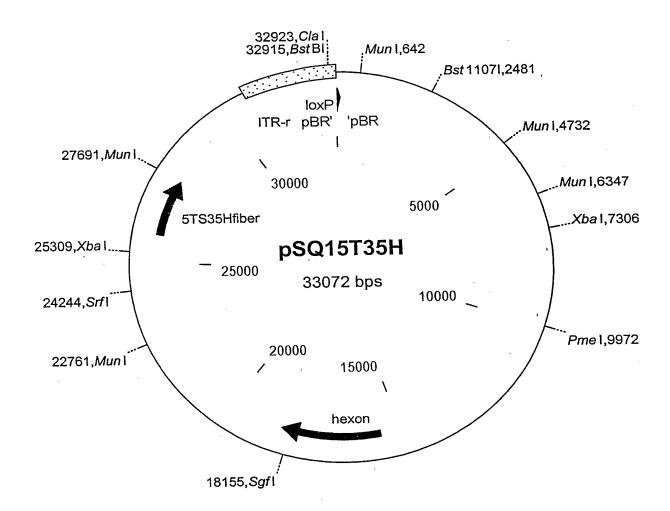
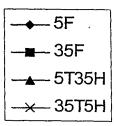


FIG. 18B



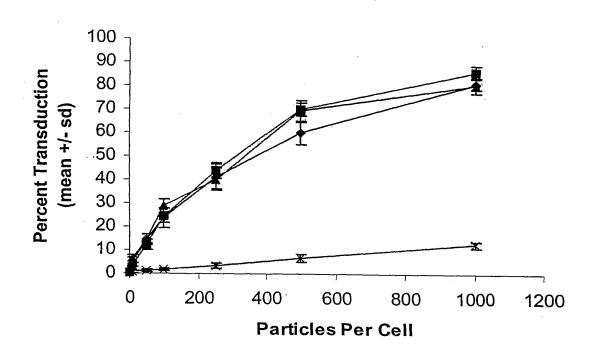


FIG. 19

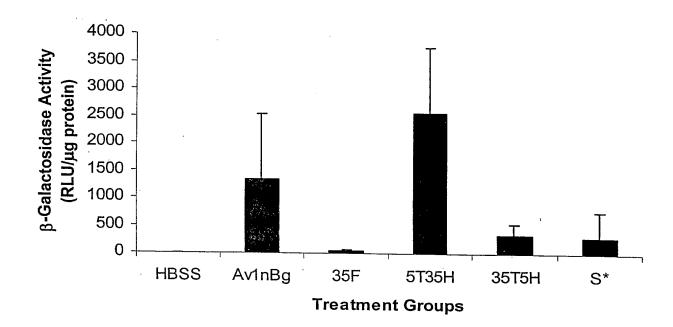


FIG. 20

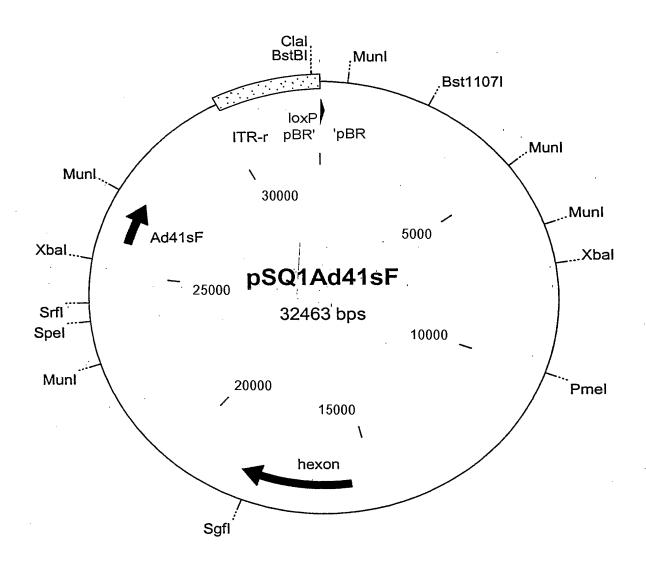


FIG. 21A

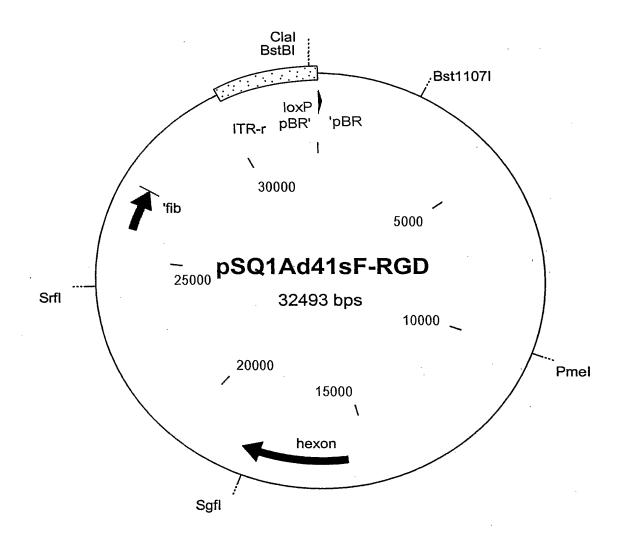
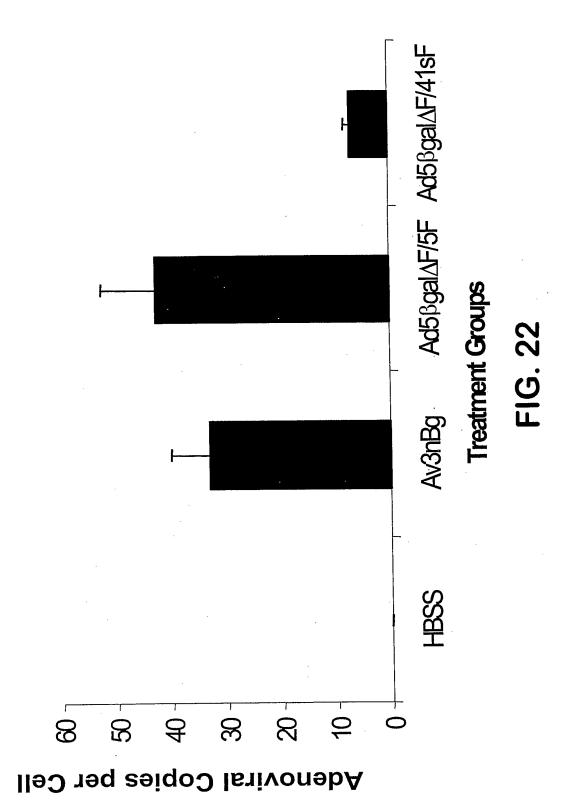
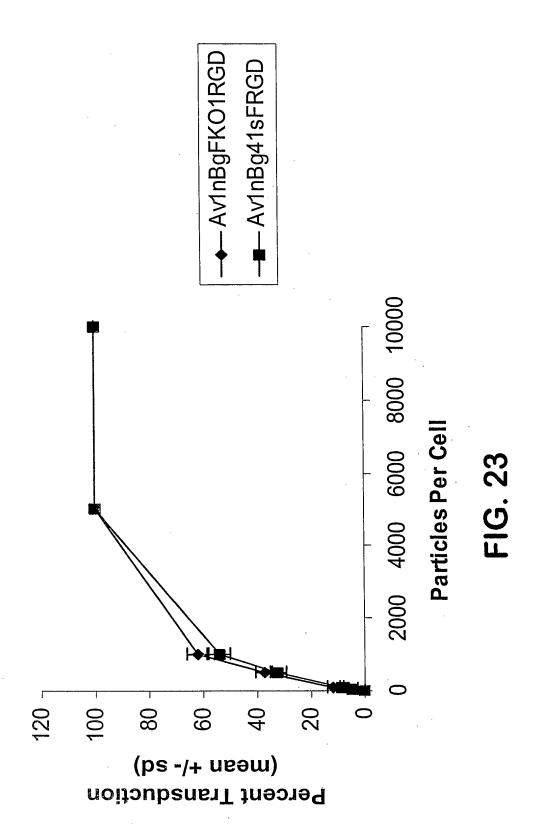
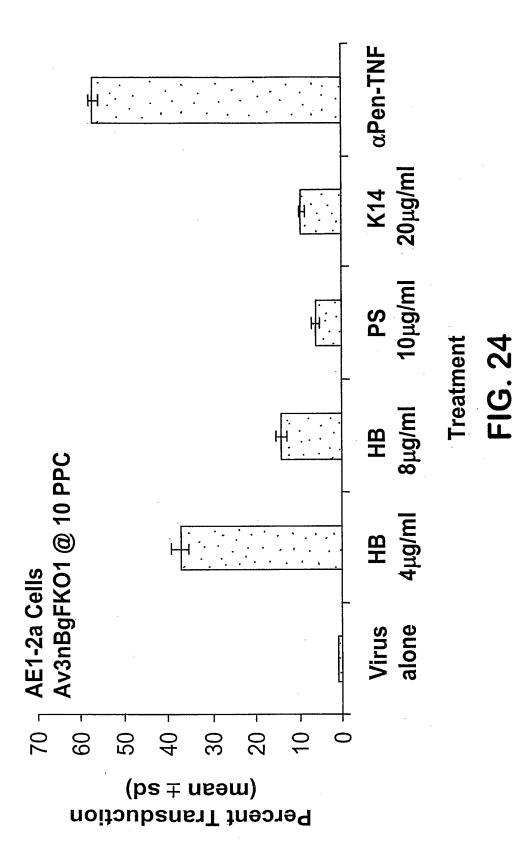


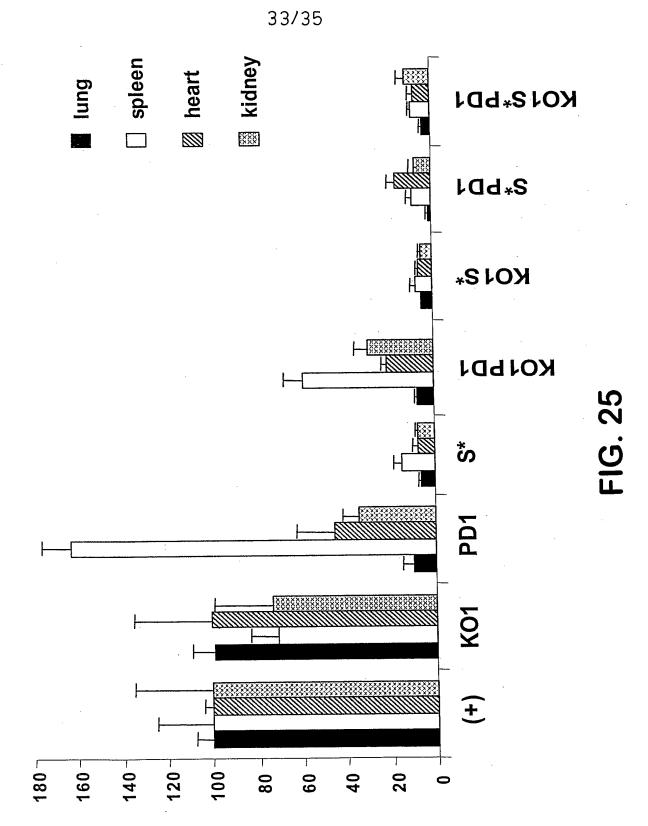
FIG. 21B



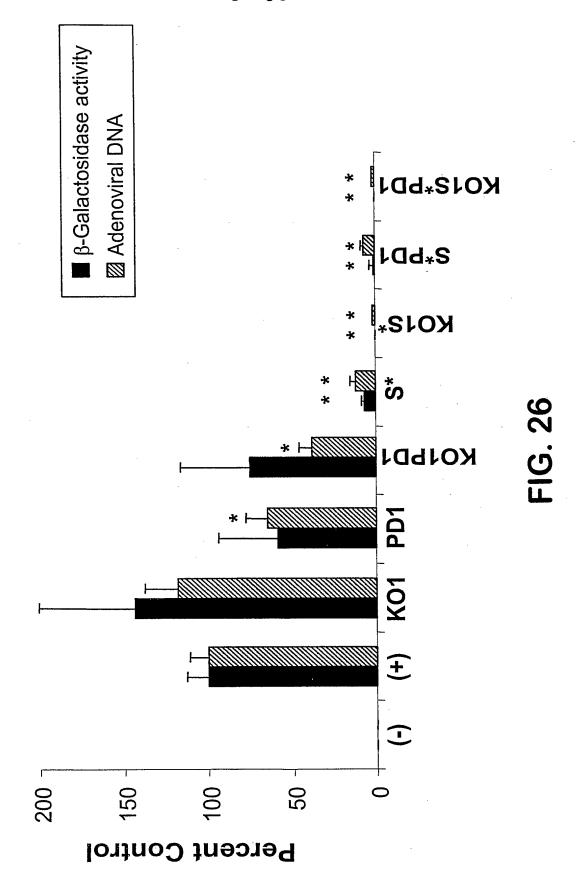
PCT/US2004/009219 WO 2004/099422

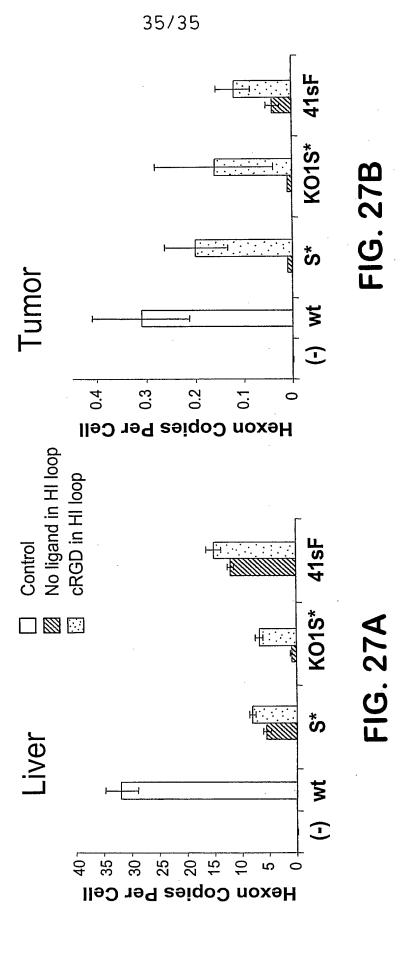






Percent Control





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SEQUENCE LISTING

<110> The Scripps Research Institute
 Von Seggern, Daniel J.

100

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| | gtg Val | | | | | | | | | | | | | | | 384 |
|------------|-------------------|-------------------|------------|-------------------|------------|-------------------|-------------------|------------|-------------------|------------|-------------------|-------------------|------------|-------------------|------------|------|
| | caa Gln 130 | | | | | | | | | | | | | | | 432 |
| | acc Thr | | | | | | | | | | | | | | | 480 |
| | tca Ser | | | | | | | | | | | | | | | 528 |
| | tca Ser | | | | | | | | | | | | | | | 576 |
| aaa Lys | gag Glu | ccc Pro 195 | att Ile | tat Tyr | aca Thr | caa Gln | aat Asn 200 | gga Gly | aaa Lys | cta Leu | gga Gly | cta Leu 205 | aag Lys | tac Tyr | glà aaa | 624 |
| gct Ala | cct Pro 210 | ttg Leu | cat His | gta Val | aca Thr | gac Asp 215 | gac Asp | cta Leu | aac Asn | act Thr | ttg Leu 220 | acc Thr | gta Val | gca Ala | act Thr | 672 |
| | cca Pro | | | | | | | | | | | | | | | 720 |
| | gcc Ala | | | | | | | | | | | | | | | 768 |
| | gga Gly | | | | | | | | | | | | | | | 816 |
| agt Ser | tat Tyr | ccg Pro 275 | ttt Phe | gat Asp | gct Ala | caa Gln | aac Asn 280 | caa Gln | cta Leu | aat Asn | cta Leu | aga Arg 285 | cta Leu | gga Gly | cag Gln | 864 |
| | cct Pro 290 | | | | | | | | | | | | | | | 912 |
| | ggc Gly | | | | | | | | | | | | | | | 960 |
| gtt Val | aac Asn | cta Leu | agc Ser | act Thr 325 | gcc Ala | aag Lys | gly aaa | ttg Leu | atg Met 330 | ttt Phe | gac Asp | gct Ala | aca Thr | gcc Ala 335 | ata Ile | 1008 |
| | att Ile | | | | | | | | | | | | | | | 1056 |
| aac | aca | aat | CCC | ctc | aaa | aca | aaa | att | ggc | cat | ggc | cta | gaa | ttt | gat | 1104 |

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| Asn | Thr | Asn 355 | Pro | Leu | Lys | Thr | Lys 360 | Ile | Gly | His | Gly | Leu 365 | Glu | Phe | Asp | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| tca Ser | aac Asn 370 | aag Lys | gct Ala | atg Met | gtt Val | cct Pro 375 | aaa Lys | cta Leu | gga Gly | act Thr | ggc 380 | ctt Leu | agt Ser | ttt Phe | gac Asp | 1152 |
| agc Ser 385 | aca Thr | ggt Gly | gcc Ala | att Ile | aca Thr 390 | gta Val | gga Gly | aac Asn | aaa Lys | aat Asn 395 | aat Asn | gat Asp | aag Lys | cta Leu | act Thr 400 | 1200 |
| ttg Leu | tgg Trp | acc Thr | aca Thr | cca Pro 405 | gct Ala | cca Pro | tct Ser | cct Pro | aac Asn 410 | tgt Cys | aga Arg | cta Leu | aat Asn | gca Ala 415 | gag Glu | 1248 |
| aaa Lys | gat Asp | gct Ala | aaa Lys 420 | ctc Leu | act Thr | ttg Leu | gtc Val | tta Leu 425 | aca Thr | aaa Lys | tgt Cys | ggc Gly | agt Ser 430 | caa Gln | ata Ile | 1296 |
| ctt Leu | gct Ala | aca Thr 435 | gtt Val | tca Ser | gtt Val | ttg Leu | gct Ala 440 | gtt Val | aaa Lys | ggc Gly | agt Ser | ttg Leu 445 | gct Ala | cca Pro | ata Ile | 1344 |
| tct Ser | gga Gly 450 | aca Thr | gtt Val | caa Gln | agt Ser | gct Ala 455 | cat His | ctt Leu | att Ile | ata Ile | aga Arg 460 | ttt Phe | gac Asp | gaa Glu | aat Asn | 1392 |
| gga Gly 465 | gtg Val | cta Leu | cta Leu | aac Asn | aat Asn 470 | tcc Ser | ttc Phe | ctg Leu | gac Asp | cca Pro 475 | gaa Glu | tat Tyr | tgg Trp | aac Asn | ttt Phe 480 | 1440 |
| aga Arg | aat Asn | gga Gly | gat Asp | ctt Leu 485 | act Thr | gaa Glu | Gly ggc | aca Thr | gcc Ala 490 | tat Tyr | aca Thr | aac Asn | gct Ala | gtt Val 495 | gga Gly | 1488 |
| ttt Phe | atg Met | cct Pro | aac Asn 500 | cta Leu | tca Ser | gct Ala | tat Tyr | cca Pro 505 | aaa Lys | tct Ser | cac His | ggt Gly | aaa Lys 510 | act Thr | gcc Ala | 1536 |
| aaa Lys | agt Ser | aac Asn 515 | Ile | gtc Val | agt Ser | caa Gln | gtt Val 520 | tac Tyr | tta Leu | aac Asn | gga Gly | gac Asp 525 | aaa Lys | act Thr | aaa Lys | 1584 |
| cct Pro | gta Val 530 | aca Thr | cta Leu | acc Thr | att Ile | aca Thr 535 | cta Leu | aac Asn | ggt Gly | aca Thr | cag Gln 540 | Glu | aca Thr | gga Gly | gac Asp | 1632 |
| aca Thr 545 | | cca Pro | agt Ser | gca Ala | tac Tyr 550 | Ser | atg Met | tca Ser | ttt Phe | tca Ser 555 | Trp | gac Asp | tgg Trp | tct Ser | ggc Gly 560 | 1680 |
| cac Hìs | aac Asn | tac Tyr | att Ile | aat Asn 565 | . Glu | ata Ile | ttt Phe | gcc Ala | aca Thr 570 | Ser | tct Ser | tac Tyr | act Thr | ttt Phe 575 | tca Ser | 1728 |
| tac Tyr | att Ile | gcc Ala | caa Gln 580 | Glu | taa * | | | | | | | | | | | 1746 |

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<211> 580 <212> PRT <213> Adenovirus type 5 <400> 2 Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro Tyr 1.0 Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro Phe 2.5 20 Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser Leu 35 40 Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu Lys 55 Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser Gln 75 Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn Ile 85 90 Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu Thr 1.00 1.05 110 Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr Met 115 120 125 Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile Ala 135 140 Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln Thr 150 155 Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr Ala 170 Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu Lys 180 185 190 Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly Ala 195 200 205 Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr Gly 215 220 Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr Gly 230 235 240 Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala Gly 250 245 255 Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val Ser 260 265 . 270 Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln Gly 280 Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn Lys 295 300 Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Leu Glu Val 310 315 320 Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile Ala 325 330 Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro Asn 340 345 350 Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp Ser 355 360 365 Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp Ser 370 375 380 Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr Leu 390 395 Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu Lys 405 410 415 Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile Leu 420 425 430 Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile Ser

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Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly
                       455
                                               460
Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg
                     470
                                           475
Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe
                 485
                                       490
                                                             495
Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys
             500
                                   505
                                                        510
Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro
         515
                              520
                                                    525
Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr
                          535
                                               540
Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His
                      550
                                        555
                                                                 560
Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr
                                       570
                 565
                                                             575
Ile Ala Gln Glu
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                                                                        48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
                                        10
tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act ccc CCC Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
              20
ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc
                                                                        192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc
                                                                        240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
                  85
ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
                                                                        336
                                   105
act gtg gct gcc gca cct cta atg gtc gcg ggc aac aca ctc acc
```

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| Thr | Val | Ala 115 | Ala | Ala | Ala | Pro | Leu 120 | Met | Val | Ala | Gly | Asn 125 | Thr | Leu | Thr | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------------|------|
| atg Met | caa Gln 130 | tca Ser | cag Gln | gcc Ala | ccg Pro | cta Leu 135 | acc Thr | gtg Val | cac His | gac Asp | tcc Ser 140 | aaa Lys | ctt Leu | agc Ser | att Ile | 432 |
| gcc Ala 145 | acc Thr | caa Gln | gga Gly | ccc Pro | ctc Leu 150 | aca Thr | gtg Val | tca Ser | gaa Glu | gga Gly 155 | aag Lys | cta Leu | gcc Ala | ctg Leu | caa Gln 160 | 480 |
| aca Thr | tca Ser | ggc | ccc Pro | ctc Leu 165 | acc Thr | acc Thr | acc Thr | gat Asp | agc Ser 170 | agt Ser | acc Thr | ctt Leu | act Thr | atc Ile 175 | act Thr | 528 |
| gcc Ala | tca Ser | ccc Pro | cct Pro 180 | cta Leu | act Thr | act Thr | gcc Ala | act Thr 185 | ggt Gly | agc Ser | ttg Leu | Gly | att Ile 190 | gac Asp | ttg Leu | 576 |
| aaa Lys | gag Glu | ccc Pro 195 | att Ile | tat Tyr | aca Thr | caa Gln | aat Asn 200 | gga Gly | aaa Lys | cta Leu | gga Gly | cta Leu 205 | aag Lys | tac Tyr | gl ^à aaa | 624 |
| gct Ala | cct Pro 210 | ttg Leu | cat His | gta Val | aca Thr | gac Asp 215 | gac Asp | cta Leu | aac Asn | act Thr | ttg Leu 220 | acc Thr | gta Val | gca Ala | act Thr | 672 |
| ggt Gly 225 | cca Pro | ggt Gly | gtg Val | act Thr | att Ile 230 | aat Asn | aat Asn | act Thr | tcc Ser | ttg Leu 235 | caa Gln | act Thr | aaa Lys | gtt Val | act Thr 240 | 720 |
| gga Gly | gcc Ala | ttg Leu | ggt Gly | ttt Phe 245 | gat Asp | tca Ser | caa Gln | ggc Gly | aat Asn 250 | atg Met | caa Gln | ctt Leu | aat Asn | gta Val 255 | gca Ala | 768 |
| gga Gly | gga Gly | cta Leu | agg Arg 260 | att Ile | gat Asp | tct Ser | caa Gln | aac Asn 265 | aga Arg | cgc Arg | ctt Leu | ata Ile | ctt Leu 270 | gat Asp | gtt Val | 816 |
| agt Ser | tat Tyr | ccg Pro 275 | ttt Phe | gat Asp | gct Ala | caa Gln | aac Asn 280 | caa Gln | cta Leu | aat Asn | cta Leu | aga Arg 285 | cta Leu | gga Gly | cag Gln | 864 |
| ggc Gly | cct Pro 290 | ctt Leu | ttt Phe | ata Ile | aac Asn | tca Ser 295 | gcc Ala | cac His | aac Asn | ttg Leu | gat Asp 300 | att Ile | aac Asn | tac Tyr | aac Asn | 912 |
| aaa Lys 305 | gly ggc | ctt Leu | tac Tyr | ttg Leu | ttt Phe 310 | aca Thr | gct Ala | tca Ser | aac Asn | aat Asn 315 | tcc Ser | aaa Lys | aag Lys | ctt Leu | gag Glu 320 | 960 |
| gtt Val | aac Asn | cta Leu | agc Ser | act Thr 325 | gcc Ala | aag Lys | gly aaa | ttg Leu | atg Met 330 | ttt Phe | gac Asp | gct Ala | aca Thr | gcc Ala 335 | ata Ile | 1008 |
| gcc Ala | att Ile | aat Asn | gca Ala 340 | gga Gly | gat Asp | gly aaa | ctt Leu | gaa Glu 345 | ttt Phe | ggt Gly | tca Ser | cct Pro | aat Asn 350 | gca Ala | cca Pro | 1056 |
| aac Asn | aca Thr | aat Asn | ccc Pro | ctc Leu | aaa Lys | aca Thr | aaa Lys | att Ile | ggc Gly | cat His | ggc Gly | cta Leu | gaa Glu | ttt Phe | gat Asp | 1104 |

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| | | 355 | | | | | 360 | | | | | 365 | | | | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| tca Ser | aac Asn 370 | aag Lys | gct Ala | atg Met | gtt Val | cct Pro 375 | aaa Lys | cta Leu | gga Gly | act Thr | ggc 380 | ctt Leu | agt Ser | ttt Phe | gac Asp | 1152 |
| agc Ser 385 | aca Thr | ggt Gly | gcc Ala | att Ile | aca Thr 390 | gta Val | gga Gly | aac Asn | aaa Lys | aat Asn 395 | aat Asn | gat Asp | aag Lys | cta Leu | act Thr 400 | 1200 |
| ttg Leu | tgg Trp | acc Thr | aca Thr | cca Pro 405 | gct Ala | cca Pro | gag Glu | gct Ala | aac Asn 410 | tgt Cys | aga Arg | cta Leu | aat Asn | gca Ala 415 | gag Glu | 1248 |
| aaa Lys | gat Asp | gct Ala | aaa Lys 420 | ctc Leu | act Thr | ttg Leu | gtc Val | tta Leu 425 | aca Thr | aaa Lys | tgt Cys | ggc Gly | agt Ser 430 | caa Gln | ata Ile | 1296 |
| ctt Leu | gct Ala | aca Thr 435 | gtt Val | tca Ser | gtt Val | ttg Leu | gct Ala 440 | gtt Val | aaa Lys | gly ggc | agt Ser | ttg Leu 445 | gct Ala | cca Pro | ata Ile | 1344 |
| tct Ser | gga Gly 450 | aca Thr | gtt Val | caa Gln | agt Ser | gct Ala 455 | cat His | ctt Leu | att Ile | ata Ile | aga Arg 460 | ttt Phe | gac Asp | gaa Glu | aat Asn | 1392 |
| gga Gly 465 | gtg Val | cta Leu | cta Leu | aac Asn | aat Asn 470 | tcc Ser | ttc Phe | ctg Leu | gac Asp | cca Pro 475 | gaa Glu | tat Tyr | tgg Trp | aac Asn | ttt Phe 480 | 1440 |
| aga Arg | aat Asn | gga Gly | gat Asp | ctt Leu 485 | act Thr | gaa Glu | ggc Gly | aca Thr | gcc Ala 490 | tat Tyr | aca Thr | aac Asn | gct Ala | gtt Val 495 | gga Gly | 1488 |
| ttt Phe | atg Met | cct Pro | aac Asn 500 | cta Leu | tca Ser | gct Ala | tat Tyr | cca Pro 505 | aaa Lys | tct Ser | cac His | ggt Gly | aaa Lys 510 | act Thr | gcc Ala | 1536 |
| aaa Lys | agt Ser | aac Asn 515 | Ile | gtc Val | agt Ser | caa Gln | gtt Val 520 | tac Tyr | tta Leu | aac Asn | gga Gly | gac Asp 525 | aaa Lys | act Thr | aaa Lys | 1584 |
| cct Pro | gta Val 530 | Thr | cta Leu | acc Thr | att Ile | aca Thr 535 | Leu | aac Asn | ggt Gly | aca Thr | cag Gln 540 | Glu | aca Thr | gga Gly | gac Asp | 1632 |
| aca Thr 545 | Thr | cca Pro | agt Ser | gca Ala | tac Tyr 550 | tct Ser | atg Met | tca Ser | ttt Phe | tca Ser 555 | Trp | gac Asp | tgg Trp | tct Ser | ggc Gly 560 | 1680 |
| cac His | aac Asn | tac Tyr | att Ile | aat Asn 565 | Glu | ata Ile | ttt Phe | gcc Ala | aca Thr 570 | Ser | tct Ser | tac Tyr | act Thr | ttt Phe 575 | tca Ser | 1728 |
| | | | caa Gln 580 | Glu | | | | | | | | | | | | 1746 |

<210> 4 <211> 581

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<212> PRT <213> Artificial Sequence <220> <223> 5F KO1 <400> 4 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro 10 5 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro 25 20 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser 45 4.0 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu 55 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser 75 70 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn 90 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu 110 105 100 Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr 125 120 Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile 135 Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln 155 150 Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr 165 Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu 180 185 190 Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205 195 200 Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr 220 215 Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr 235 230 Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala 245 250 255 Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val 260 265 Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln 280 285 Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn 300 295 Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu 310 315 Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile 325 330 335 Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro 345 340 Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp 360 Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp 380 375 Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr 395 390 Leu Trp Thr Thr Pro Ala Pro Glu Ala Asn Cys Arg Leu Asn Ala Glu 410 415 Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile 425

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Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile 435 440 Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn 450 455 460 Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe 470 475 Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly 490 485 Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala 500 505 510 Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys 520 Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp 535 Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly 550 555 His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser 565 Tyr Ile Ala Gln Glu 580 <210> 5 <211> 1776 <212> DNA <213> Artificial Sequence <220> <223> 5F KO1RGD <221> CDS <222> (1)...(1746) atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct 144 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu 192 aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu 100 105

| act Thr | gtg Val | gct Ala 115 | gcc Ala | gcc Ala | gca Ala | cct Pro | cta Leu 120 | atg Met | gtc Val | gcg Ala | ggc Gly | aac Asn 125 | aca Thr | ctc Leu | acc Thr | 384 |
|-------------------|-------------------|-------------------|------------|------------|-------------------|-------------------|-------------------|------------|------------|-------------------|-------------------|-------------------|------------|------------|-------------------|------|
| atg Met | caa Gln 130 | tca Ser | cag Gln | gcc Ala | ccg Pro | cta Leu 135 | acc Thr | gtg Val | cac His | gac Asp | tcc Ser 140 | aaa Lys | ctt Leu | agc Ser | att Ile | 432 |
| | | | | | ctc Leu 150 | | | | | | | | | | | 480 |
| | | | | | acc Thr | | | | | | | | | | | 528 |
| | | | | | act Thr | | | | | | | | | | | 576 |
| | | | | | aca Thr | | | | | | | | | | | 624 |
| | | | | | aca Thr | | | | | | | | | | | 672 |
| ggt Gly 225 | cca Pro | ggt Gly | gtg Val | act Thr | att Ile 230 | aat Asn | aat Asn | act Thr | tcc Ser | ttg Leu 235 | caa Gln | act Thr | aaa Lys | gtt Val | act Thr 240 | 720 |
| | | | | | gat Asp | | | | | | | | | | | 768 |
| | | | | | gat Asp | | | | | | | | | | | 816 |
| | | | | | gct Ala | | | | | | | | | | | 864 |
| ggc | cct Pro 290 | ctt Leu | ttt Phe | ata Ile | aac Asn | tca Ser 295 | gcc Ala | cac His | aac Asn | ttg Leu | gat Asp 300 | att Ile | aac Asn | tac Tyr | aac Asn | 912 |
| | | | | | ttt Phe 310 | | | | | | | | | | | 960 |
| | | | | | gcc Ala | | | | | | | | | | | 1008 |
| | | | | | gat Asp | | | | | | | | | | | 1056 |

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| | | | | | | aca Thr | | | | | | | | | | 1104 |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| | | | | | | cct Pro 375 | | | | | | | | | | 1152 |
| agc Ser 385 | aca Thr | ggt Gly | gcc Ala | att Ile | aca Thr 390 | gta Val | gga Gly | aac Asn | aaa Lys | aat Asn 395 | aat Asn | gat Asp | aag Lys | cta Leu | act Thr 400 | 1200 |
| ttg Leu | tgg Trp | acc Thr | aca Thr | cca Pro 405 | gct Ala | cca Pro | tct Ser | cct Pro | aac Asn 410 | tgt Cys | aga Arg | cta Leu | aat Asn | gca Ala 415 | gag Glu | 1248 |
| aaa Lys | gat Asp | gct Ala | aaa Lys 420 | ctc Leu | act Thr | ttg Leu | gtc Val | tta Leu 425 | aca Thr | aaa Lys | tgt Cys | ggc | agt Ser 430 | caa Gln | ata Ile | 1296 |
| ctt Leu | gct Ala | aca Thr 435 | gtt Val | tca Ser | gtt Val | ttg Leu | gct Ala 440 | gtt Val | aaa Lys | ggc Gly | agt Ser | ttg Leu 445 | gct Ala | cca Pro | ata Ile | 1344 |
| tct Ser | gga Gly 450 | aca Thr | gtt Val | caa Gln | agt Ser | gct Ala 455 | cat His | ctt Leu | att Ile | ata Ile | aga Arg 460 | ttt Phe | gac Asp | gaa Glu | aat Asn | 1392 |
| gga Gly 465 | gtg Val | cta Leu | cta Leu | aac Asn | aat Asn 470 | tcc Ser | ttc Phe | ctg Leu | gac Asp | cca Pro 475 | gaa Glu | tat Tyr | tgg Trp | aac Asn | ttt Phe 480 | 1440 |
| aga Arg | aat Asn | gga Gly | gat Asp | ctt Leu 485 | act Thr | gaa Glu | gly ggc | aca Thr | gcc Ala 490 | tat Tyr | aca Thr | aac Asn | gct Ala | gtt Val 495 | gga Gly | 1488 |
| ttt Phe | atg Met | cct Pro | aac Asn 500 | cta Leu | tca Ser | gct Ala | tat Tyr | cca Pro 505 | aaa Lys | tct Ser | cac His | ggt Gly | aaa Lys 510 | act Thr | gcc Ala | 1536 |
| aaa Lys | agt Ser | aac Asn 515 | att Ile | gtc Val | agt Ser | caa Gln | gtt Val 520 | tac Tyr | tta Leu | aac Asn | gga Gly | gac Asp 525 | aaa Lys | act Thr | aaa Lys | 1584 |
| cct Pro | gta Val 530 | aca Thr | cta Leu | acc Thr | att Ile | aca Thr 535 | cta Leu | aac Asn | ggt Gly | aca Thr | cag Gln 540 | gaa Glu | aca Thr | ggt Gly | gat Asp | 1632 |
| cat His 545 | tgt Cys | gat Asp | tgt Cys | cgt Arg | ggt Gly 550 | gat Asp | tgt Cys | ttt Phe | tgt Cys | aca Thr 555 | act Thr | cca Pro | agt Ser | gca Ala | tac Tyr 560 | 1680 |
| tct Ser | atg Met | tca Ser | ttt Phe | tca Ser 565 | tgg Trp | gac Asp | tgg Trp | tct Ser | ggc Gly 570 | cac His | aac Asn | tac Tyr | att Ile | aat Asn 575 | gaa Glu | 1728 |
| ata Ile | ttt Phe | gcc Ala | aca Thr 580 | tcc Ser | tct Ser | taca | icttt | tt c | atac | attg | la ac | aaga | ıataa | ı | | 1776 |

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```
<210> 6
<211> 582
<212> PRT
<213> Artificial Sequence
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<223> 5F KO1RGD
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                                 10
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
          20
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
                         40
                                           45
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
                   55
                                      60
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
                  70
                                    75
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
                              90
              8.5
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
                             105
Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
                          120
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
                    135
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
                150
                                    155
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
           165
                                170
                                                    175
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
                        200
                                            205
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
                     215
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
                  230
                                    235
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
                              250
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
                          265 270
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
                       280
                                            285
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
                     295
                                       300
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
                 310
                                    315
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
              325
                                330
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
                             345
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
                         360
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
                      375
                                        380
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
               390
                          395 400
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
                                410
```

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| Lys | Asp | Ala | Lys 420 | Leu | Thr | Leu | Val | Leu 425 | Thr | Lys | Cys | Gly | Ser 430 | Gln | Ile | |
|--------------|---------------------------------|------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|
| Leu | Ala | Thr 435 | | Ser | Val | Leu | Ala 440 | | Lys | Gly | Ser | Leu 445 | Ala | Pro | Ile | |
| Ser | Gly 450 | Thr | Val | Gln | Ser | Ala 455 | His | Leu | Ile | Ile | Arg 460 | Phe | Asp | Glu | Asn | |
| Gly 465 | Val | Leu | Leu | Asn | Asn 470 | Ser | Phe | Leu | Asp | Pro 475 | Glu | Tyr | Trp | Asn | Phe 480 | |
| Arg | Asn | Gly | qaA | Leu 485 | Thr | Glu | Gly | Thr | Ala 490 | Tyr | Thr | Asn | Ala | Val 495 | Gly | |
| Phe | Met | Pro | Asn 500 | Leu | Ser | Ala | Tyr | Pro 505 | Lys | Ser | His | Gly | Lys 510 | Thr | Ala | |
| Lys | Ser | Asn 515 | Ile | Val | Ser | Gln | Val 520 | Tyr | Leu | Asn | Gly | Asp 525 | Lys | Thr | Lys | |
| | Val 530 | | | | | 535 | | | | | 540 | | | | | |
| His 545 | Cys | Asp | Cys | Arg | Gly 550 | Asp | Cys | Phe | Cys | Thr 555 | Thr | Pro | Ser | Ala | Tyr 560 | |
| Ser | Met | Ser | Phe | Ser 565 | Trp | Asp | Trp | Ser | Gly 570 | His | Asn | Tyr | Ile | Asn 575 | Glu | |
| Ile | Phe | Ala | Thr 580 | Ser | Ser | | | | | | | | | | | |
| <213 <213 | 0> 7 l> 17 2> DN 3> An | JA. | lcial | l Sec | quenc | ce | | | | | | | | | | |
| <220 <220 | 0> 3> 51 | F KOI | L2 | | | | | | | | | | | | | |
| | l> CI 2> (1 | | .(174 | 16) | | | | | | | | | | | | |
| atg | 0> 7 aag | | | | | | | | | | | | | | | 48 |
| Met 1 | Lys | Arg | Ala | Arg 5 | Pro | Ser | Glu | Asp | Thr 10 | Phe | Asn | Pro | Val | Tyr 15 | Pro | |
| | gac Asp | | | | | | | | | | | | | | | 96 |
| ttt Phe | gta Val | tcc Ser 35 | Pro | Asn | Gly | Phe | ${	t Gln}$ | Glu | Ser | Pro | cct Pro | Gly | Val | ctc Leu | tct Ser | 144 |
| | cgc Arg 50 | | | | | | | | | | | | | | | 192 |
| | atg Met | | | | | | | | | | | | | | | 240 |
| | aat Asn | | | | | | | | Leu | | | | | | | 288 |
| | | | | 85 | | | | | 90 | | | | | 95 | | |

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| Ile | Asn | Leu | Glu 100 | Ile | Ser | Ala | Pro | Leu 105 | Thr | Val | Thr | Ser | Glu 110 | Ala | Leu | |
|-------------------|------------|------------|------------|-------------------|-------------------|------------|------------|------------|------------|-------------------|------------|------------|------------|------------|-------------------|------|
| | | | | gcc Ala | | | | | | | | | | | | 384 |
| | | | | gcc Ala | | | | | | | | | | | | 432 |
| gcc Ala 145 | acc Thr | caa Gln | gga Gly | ccc Pro | ctc Leu 150 | aca Thr | gtg Val | tca Ser | gaa Glu | gga Gly 155 | aag Lys | cta Leu | gcc Ala | ctg Leu | caa Gln 160 | 480 |
| | | | | ctc Leu 165 | | | | | | | | | | | | 528 |
| | | | | cta Leu | | | | | | | | | | | | 576 |
| | | | | tat Tyr | | | | | | | | | | | | 624 |
| | | | | gta Val | | | | | | | | | | | | 672 |
| | | | | act Thr | | | | | | | | | | | | 720 |
| | | | | ttt Phe 245 | | | | | | | | | | | | 768 |
| | | | | att Ile | | | | | | | | | | | | 816 |
| | | | | gat Asp | | | | | | | | | | | | 864 |
| | | | | ata Ile | | | | | | | | | | | | 912 |
| | | | | ttg Leu | | | | | | | | | | | | 960 |
| | | | | act Thr 325 | | | | | | | | | | | | 1008 |
| | | | | gga Gly | | | | | | | | | | | | 1056 |

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| | 340 | | | | | 345 | | | | | 350 | | | |
|-------------------------------|---------------------------|----------------|------------|-------------------|------------|-------------------|------------|------------|-------------------|------------|-------------------|------------|------------|------|
| aac aca a Asn Thr A 3 | | | | | | | | | | | | | | 1104 |
| tca aac a Ser Asn I 370 | | | | | | | | | | | | | | 1152 |
| agc aca g Ser Thr G 385 | | Ile ' | | | | | | | | | | | | 1200 |
| ttg tgg a Leu Trp I | | | | | | | | | | | | | | 1248 |
| gga gat g Gly Asp A | gct aaa Ala Lys 420 | ctc : Leu ' | act Thr | ttg Leu | gtc Val | tta Leu 425 | aca Thr | aaa Lys | tgt Cys | ggc Gly | agt Ser 430 | caa Gln | ata Ile | 1296 |
| ctt gct a Leu Ala I 4 | | | | | | | | | | | | | | 1344 |
| tct gga a Ser Gly I 450 | | | Ser | | | | | | | | | | | 1392 |
| gga gtg c Gly Val I 465 | | Asn 2 | | | | | | | | | | | | 1440 |
| aga aat g Arg Asn G | | | | | | | | | | | | | | 1488 |
| ttt atg c Phe Met P | | | | | | | | | | | | | | 1536 |
| aaa agt a Lys Ser A 5 | | | | | | | | | | | | | | 1584 |
| cct gta a Pro Val T 530 | aca cta Thr Leu | acc : Thr : | Ile | aca Thr 535 | cta Leu | aac Asn | ggt Gly | aca Thr | cag Gln 540 | gaa Glu | aca Thr | gga Gly | gac Asp | 1632 |
| aca act c Thr Thr P 545 | | Āla | | | | | | | | | | | | 1680 |
| cac aac t His Asn T | | | | | | | | | | | | | | 1728 |
| tac att g Tyr Ile A | · | | taa * | | | | | | | | | | | 1746 |

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```
<210> 8
<211> 581
<212> PRT
<213> Artificial Sequence
<220>
<223> 5F KO12
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                                              15
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
                           25
          2.0
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
      35
                       40
                                         45
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
 50
                    55
                                     60
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
                 70
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
             85
                             90
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
         100
                         105 110
Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
    115
           120
                                         125
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
                    135
   130
                                      140
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
       150 155
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
                               170
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
                          185
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
                     200
      195
                                         205
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
 210
                  215 220
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
                230 235
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
             245
                               250
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
         260
                          265
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
                      280
      275
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
        295
                                   300
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305
              310
                                  315
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
             325
                               330
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
                           345
         340
                                          350
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
      355
                        360
                                         365
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
                    375
                            380
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
                 390
                                  395
```

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| Leu Trp | | | | | | | | | | | | | | | |
|---|--|--|---|--|---|---|--|---|--|--|--|--|---|--|-------------------------|
| LCG IIP | Thr | Thr | Pro 405 | Ala | Pro | Ser | Pro | Asn 410 | Cys | Ser | Leu | Asn | Gly 415 | Gly | |
| Gly Asp | Ala | Lys 420 | Leu | Thr | Leu | Val | Leu 425 | Thr | Lys | Сув | Gly | Ser 430 | Gln | Ile | |
| Leu Ala | Thr 435 | Val | Ser | Val | Leu | Ala 440 | Val | Lys | Gly | Ser | Leu 445 | | Pro | Ile | |
| Ser Gly 450 | | Val | Gln | Ser | Ala 455 | | Leu | Ile | Ile | Arg 460 | | Asp | Glu | Asn | |
| Gly Val | Leu | Leu | Asn | Asn 470 | | Phe | Leu | Asp | Pro 475 | | Tyr | Trp | Asn | Phe 480 | |
| Arg Asn | Gly | Asp | Leu 485 | | Glu | Gly | Thr | Ala 490 | | Thr | Asn | Ala | Val 495 | | |
| Phe Met | Pro | Asn 500 | | Ser | Ala | Tyr | Pro 505 | | Ser | His | Gly | Lys 510 | | Ala | |
| Lys Ser | Asn 515 | | Val | Ser | Gln | Val 520 | | Leu | Asn | Gly | Asp 525 | | Thr | Lys | |
| Pro Val 530 | | Leu | Thr | Ile | Thr 535 | | Asn | Gly | Thr | Gln 540 | | Thr | Gly | Asp | |
| Thr Thr | Pro | Ser | Ala | Tyr 550 | | Met | Ser | Phe | Ser 555 | | Asp | Trp | Ser | Gly 560 | |
| His Asn | Tyr | Ile | Asn 565 | | Ile | Phe | Ala | | | Ser | Tyr | Thr | | | |
| Tyr Ile | Ala | Gln 580 | _ | | | | | 570 | | | | | 575 | | |
| | | 560 | | | | | | | | | | | | | |
| <210> 9 | | | | | | | | | | | | | | | |
| <211> 1 | 586 | | | | | | | | | | | | | | |
| <212> DI <213> A: | | icial | . Sec | quenc | ce | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| <220× | | | | | | | | | | | | | | | |
| <220> <223> 53 | F S* | | | | | | | | | | | | | | |
| | os | .(174 | l 6) | | | | | | | | | | | | |
| <223> 53 <221> C1 | os | . (174 | 16) | | | | | | | | | | | | |
| <223> 5: <221> C! <222> (: <400> 9 acc ggt | os 1) | cca | act | gtg | cct | ttt | ctt | act | cct | ccc | ttt | gta | tcc | ccc | 48 |
| <223> 5: <221> C! <222> (: <400> 9 | os 1) | cca | act | gtg Val | cct Pro | ttt Phe | ctt Leu | act Thr 10 | cct Pro | ccc Pro | ttt Phe | gta Val | tcc Ser 15 | ccc Pro | 48 |
| <223> 53 <221> C1 <222> (3 <400> 9 acc ggt Thr Gly 1 aat ggg | cct Pro | cca Pro | act Thr 5 | Val agt | Pro | Phe | Leu ggg | Thr 10 gta | Pro | Pro tct | Phe ttg | Val | Ser 15 cta | Pro tcc | 4 8 96 |
| <223> 5: <221> CI <222> (: <400> 9 acc ggt Thr Gly 1 | cct Pro | cca Pro | act Thr 5 | Val agt | Pro | Phe | Leu ggg | Thr 10 gta | Pro | Pro tct | Phe ttg | Val | Ser 15 cta | Pro tcc | |
| <223> 5: <221> C! <222> (:) <400> 9 acc ggt Thr Gly 1 aat ggg Asn Gly | cct Pro ttt Phe | cca Pro caa Gln 20 | act Thr 5 gag Glu | Val agt Ser | Pro ccc Pro | Phe cct Pro | Leu ggg Gly 25 | Thr 10 gta Val | Pro ctc Leu | Pro tct Ser | Phe ttg Leu | Val cgc Arg 30 | Ser 15 cta Leu | Pro tcc Ser | 96 |
| <223> 53 <221> C1 <222> (3 <400> 9 acc ggt Thr Gly 1 aat ggg | cct Pro ttt Phe cta | cca Pro caa Gln 20 | act Thr 5 gag Glu | val agt ser | Pro ccc Pro | Phe cct Pro ggc Gly | ggg Gly 25 | Thr 10 gta Val | Pro ctc Leu | Pro tct Ser | Phe ttg Leu aaa Lys | Val cgc Arg 30 | Ser 15 cta Leu | Pro tcc Ser | |
| <223> 5: <221> Ci <222> (:) <400> 9 acc ggt Thr Gly 1 aat ggg Asn Gly gaa cct Glu Pro | cct Pro ttt Phe cta Leu 35 | cca Pro caa Gln 20 gtt Val | act Thr 5 gag Glu acc Thr | Val agt Ser tcc Ser | Pro ccc Pro aat Asn | Phe cct Pro ggc Gly 40 | ggg Gly 25 atg Met | Thr 10 gta Val ctt Leu | Pro ctc Leu gcg Ala | Pro tct Ser ctc Leu | Phe ttg Leu aaa Lys 45 | val cgc Arg 30 atg Met | Ser 15 cta Leu ggc Gly | tcc Ser aac Asn | 96 144 |
| <223> 53 <221> Cl <222> (3 <400> 9 acc ggt Thr Gly 1 aat ggg Asn Gly gaa cct Glu Pro ggc ctc Gly Leu | cct Pro ttt Phe cta Leu 35 | cca Pro caa Gln 20 gtt Val | act Thr 5 gag Glu acc Thr | Val agt Ser tcc Ser gag | Pro ccc Pro aat Asn | Phe cct Pro ggc Gly 40 | ggg Gly 25 atg Met | Thr 10 gta Val ctt Leu ctt | Pro ctc Leu gcg Ala acc | Pro tct Ser ctc Leu tcc | Phe ttg Leu aaa Lys 45 caa | Val cgc Arg 30 atg Met | Ser 15 cta Leu ggc Gly | tcc Ser aac Asn | 96 |
| <223> 5: <221> Cl <222> (:) <400> 9 acc ggt Thr Gly 1 aat ggg Asn Gly gaa cct Glu Pro | cct Pro ttt Phe cta Leu 35 | cca Pro caa Gln 20 gtt Val | act Thr 5 gag Glu acc Thr | Val agt Ser tcc Ser gag | Pro ccc Pro aat Asn | Phe cct Pro ggc Gly 40 | ggg Gly 25 atg Met | Thr 10 gta Val ctt Leu ctt | Pro ctc Leu gcg Ala acc | Pro tct Ser ctc Leu tcc | Phe ttg Leu aaa Lys 45 caa | Val cgc Arg 30 atg Met | Ser 15 cta Leu ggc Gly | tcc Ser aac Asn | 96 144 |
| <223> 5: <221> Cl <222> (: <400> 9 acc ggt Thr Gly 1 aat ggg Asn Gly gaa cct Glu Pro ggc ctc Gly Leu 50 act gtg | cct Pro ttt Phe cta Leu 35 tct Ser | cca Pro caa Gln 20 gtt Val ctg Leu | act Thr 5 gag Glu acc Thr gac Asp | val agt ser tcc ser gag Glu ctc | Pro ccc Pro aat Asn gcc Ala 55 | Phe cct Pro ggc Gly 40 ggc Gly | ggg Gly 25 atg Met aac Asn | Thr 10 gta Val ctt Leu ctt Leu | Pro ctc Leu gcg Ala acc Thr | Pro tct Ser ctc Leu tcc Ser 60 aac | Phe ttg Leu aaa Lys 45 caa Gln ata | Val cgc Arg 30 atg Met aat Asn | Ser 15 cta Leu ggc Gly gta Val | tcc Ser aac Asn acc Thr | 96 144 |
| <223> 5: <221> Cl <222> (: <400> 9 acc ggt Thr Gly 1 aat ggg Asn Gly gaa cct Glu Pro ggc ctc Gly Leu 50 | cct Pro ttt Phe cta Leu 35 tct Ser | cca Pro caa Gln 20 gtt Val ctg Leu | act Thr 5 gag Glu acc Thr gac Asp | val agt ser tcc ser gag Glu ctc | Pro ccc Pro aat Asn gcc Ala 55 | Phe cct Pro ggc Gly 40 ggc Gly | ggg Gly 25 atg Met aac Asn | Thr 10 gta Val ctt Leu ctt Leu | Pro ctc Leu gcg Ala acc Thr | Pro tct Ser ctc Leu tcc Ser 60 aac | Phe ttg Leu aaa Lys 45 caa Gln ata | Val cgc Arg 30 atg Met aat Asn | Ser 15 cta Leu ggc Gly gta Val | tcc Ser aac Asn acc Thr | 96 144 192 |
| <223> 5: <221> Cl <222> (:) <400> 9 acc ggt Thr Gly 1 aat ggg Asn Gly gaa cct Glu Pro ggc ctc Gly Leu 50 act gtg Thr Val 65 ata tct | cct Pro ttt Phe cta Leu 35 tct Ser agc gca | cca Pro caa Gln 20 gtt Val ctg Leu cca Pro | act Thr 5 gag Glu acc Thr gac Asp cct Pro | val agt Ser tcc Ser gag Glu ctc Leu 70 aca | Pro CCC Pro aat Asn GCC Ala 55 GGly Gtt | Phe cct Pro ggc Gly 40 ggc Gly gcc Ala | Ggg Gly 25 atg Met aac Asn gga Gly tca | Thr 10 gta Val ctt Leu ctt Leu gcc Ala | Pro ctc Leu gcg Ala acc Thr tca Ser 75 | Pro tct Ser ctc Leu tcc Ser 60 aac Asn cta | Phe ttg Leu aaa Lys 45 caa Gln ata Ile | Val cgc Arg 30 atg Met aat Asn aac | Ser 15 cta Leu ggc Gly gta Val ctg Leu | Pro tcc Ser aac Asn acc Thr gaa Glu 80 | 96 144 192 |
| <223> 5: <221> Cl <222> (:) <400> 9 acc ggt Thr Gly 1 aat ggg Asn Gly gaa cct Glu Pro ggc ctc Gly Leu 50 act gtg Thr Val 65 | cct Pro ttt Phe cta Leu 35 tct Ser agc gca | cca Pro caa Gln 20 gtt Val ctg Leu cca Pro | act Thr 5 gag Glu acc Thr gac Asp cct Pro | val agt Ser tcc Ser gag Glu ctc Leu 70 aca | Pro CCC Pro aat Asn GCC Ala 55 GGly Gtt | Phe cct Pro ggc Gly 40 ggc Gly gcc Ala | Ggg Gly 25 atg Met aac Asn gga Gly tca | Thr 10 gta Val ctt Leu ctt Leu gcc Ala | Pro ctc Leu gcg Ala acc Thr tca Ser 75 | Pro tct Ser ctc Leu tcc Ser 60 aac Asn cta | Phe ttg Leu aaa Lys 45 caa Gln ata Ile | Val cgc Arg 30 atg Met aat Asn aac | Ser 15 cta Leu ggc Gly gta Val ctg Leu | Pro tcc Ser aac Asn acc Thr gaa Glu 80 | 96 144 192 240 |

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| gcc g Ala A | | | | | | | | | | | | | | | | 336 |
|-----------------------|------------|-------------------|------------|------------|------------|------------|-------------------|------------|------------|------------|------------|-------------------|------------|------------|------------|------|
| gcc c Ala P | | | | | | | | | | | | | | | | 384 |
| ccc c Pro L 1 | | | | | | | | | | | | | | | | 432 |
| ctc a Leu T 145 | | | | | | | | | | | | | | | | 480 |
| cta a Leu T | | | | | | | | | | | | | | | | 528 |
| tat a Tyr T | | | | | | | | | | | | | | | | 576 |
| gta a Val T | aca Thr | gac Asp 195 | gac Asp | cta Leu | aac Asn | act Thr | ttg Leu 200 | acc Thr | gta Val | gca Ala | act Thr | ggt Gly 205 | cca Pro | ggt Gly | gtg Val | 624 |
| act a Thr I 2 | | | | | | | | | | | | | | | | 672 |
| ttt g Phe A 225 | | | | | | | | | | | | | | | | 720 |
| att g Ile A | | | | | | | | | | | | | | | | 768 |
| gat g Asp A | | | | | | | | | | | | | | | | 816 |
| ata a Ile A | | | | | | | | | | | | | | | | 864 |
| ttg t Leu P 2 | | | | | | | | | | | | | | | | 912 |
| act g Thr A 305 | | | | | | | | | | | | | | | | 960 |
| gga g Gly A | | | | | | | | | | | | | | | | 1008 |

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| | | | aaa Lys 340 | | | | | | | | | | | | | 1056 |
|------------|-------------------|------------|-------------------|------------|------------|-------------------|------------|------------|------------|------------|-------------------|------------|------------|------------|------------|------|
| | | | aaa Lys | | | | | | | | | | | | | 1104 |
| | | | gga Gly | | | | | | | | | | | | | 1152 |
| | | | tct Ser | | | | | | | | | | | | | 1200 |
| | | | gtc Val | | | | | | | | | | | | | 1248 |
| | | | gct Ala 420 | | | | | | | | | | | | | 1296 |
| | | | cat His | | | | | | | | | | | | | 1344 |
| | | | ttc Phe | | | | | | | | | | | | | 1392 |
| | | | gly ggc | | | | | | | | | | | | | 1440 |
| | | | tat Tyr | | | | | | | | | | | | | 1488 |
| | | | gtt Val 500 | | | | | | | | | | | | | 1536 |
| | | | cta Leu | | | | | Ğlu | | | | | Thr | | | 1584 |
| gca Ala | tac Tyr 530 | tct Ser | atg Met | tca Ser | ttt Phe | tca Ser 535 | tgg Trp | gac Asp | tgg Trp | tct Ser | ggc Gly 540 | cac His | aac Asn | tac Tyr | att Ile | 1632 |
| | | | ttt Phe | | | | | | | | | | | | | 1680 |
| gaa Glu | taa * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | 1686 |

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<210> 10 <211> 561 <212> PRT <213> Artificial Sequence <220> <223> 5F S* <400> 10 Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro Phe Val Ser Pro 1.0 Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser Leu Arg Leu Ser 25 Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu Lys Met Gly Asn 35 40 45 Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser Gln Asn Val Thr 50 55 60 Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn Ile Asn Leu Glu 70 75 Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu Thr Val Ala Ala 85 90 Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr Met Gln Ser Gln 105 Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile Ala Thr Gln Gly 115 120 125 Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln Thr Ser Gly Pro 135 140 Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr Ala Ser Pro Pro 155 150 Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu Lys Glu Pro Ile 165 170 Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly Ala Pro Leu His 180 185 Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr Gly Pro Gly Val 195 200 205 Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr Gly Ala Leu Gly 215 220 Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala Gly Gly Leu Arg 230 235 Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val Ser Tyr Pro Phe 250 245 255 Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln Gly Pro Leu Phe 260 265 Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn Lys Gly Leu Tyr 275 280 285 Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu Val Asn Leu Ser 295 Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile Ala Ile Asn Ala 310 315 Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro Asn Thr Asn Pro 325 330 Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp Ser Asn Lys Ala 340 345 350 Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp Ser Thr Gly Ala 355 360 365 Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr Leu Trp Thr Thr 375

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| 385 | Ala | | | | 390 | | | | | 395 | | | | | 400 | |
|---|---|---|--|---|--|--|--|--|---------------------------------------|---------------------------------|------------------------------------|------------------------------------|--|---|--------------------------------|-----------|
| | Thr | | | 405 | | | | | 410 | | | | | 415 | | |
| | Val | | 420 | | | | | 425 | | | | | 430 | | | |
| | Ser | 435 | | | | | 440 | | | | | 445 | | | | |
| | Asn 450 | | | | | 455 | | | | | 460 | | | | | |
| 465 | Thr | | _ | | 470 | _ | | | | 475 | | | | | 480 | |
| | Ser | | | 485 | | | | | 490 | | | | | 495 | | |
| | Ser | | 500 | | | | | 505 | | | | | 510 | | | |
| | Ile | 515 | | | _ | | 520 | | | | | 525 | | | | |
| Ala | Tyr 530 | Ser | Met | Ser | Phe | Ser 535 | Trp | Asp | Trp | Ser | Gly 540 | His | Asn | Tyr | Ile | |
| Asn 545 Glu | Glu | Ile | Phe | Ala | Thr 550 | Ser | Ser | Tyr | Thr | Phe 555 | Ser | Tyr | Ile | Ala | Gln 560 | |
| | | | | | | | | | | | | | | | | |
| <21: | 0> 1: 1> 1' 2> Di 3> A: | 776 NA | icial | l Sed | quenc | ce | | | | | | | | | | |
| <22 <22 | 0 > 3 > 51 | p c*t | | | | | | | | | | | | | | |
| | | . D | RGD | | | | | | | | | | | | | |
| | 1> CI 2> (: | os | | 16) | | | | | | | | | | | | |
| <22: | 1> CI 2> (: 0> 1: | os 1) | . (174 | | cca | tat | 422 | ga t | 200 | tta | 220 | aaa | ata | tat | cca | 4.8 |
| <22: <40 atg | 1> CI 2> (: | os 1) 1 | . (174 gca | aga | ccg Pro | tct Ser | gaa Glu | gat Asp | acc Thr 10 | ttc Phe | aac Asn | ccc Pro | gtg Val | tat Tyr 15 | cca Pro | 48 |
| <222 <40 atg Met 1 tat | 1> CI 2> (: 0> 1: aag | OS 1) 1 cgc Arg | gca Ala gaa | aga Arg 5 | Pro | Ser | Glu cca | Asp | Thr 10 gtg | Phe | Asn | Pro | Val act | Tyr 15 cct | Pro | 48 96 |
| <22: <40 atg Met 1 tat Tyr | 1> Cl 2> (: 0> 1: aag Lys | OS 1) 1 cgc Arg acg Thr | gca Ala gaa Glu 20 | aga Arg 5 acc Thr | Pro ggt Gly ggg | Ser cct Pro | Glu cca Pro | Asp act Thr 25 gag | Thr 10 gtg Val agt | Phe cct Pro | Asn ttt Phe | Pro ctt Leu ggg | Val act Thr 30 gta | Tyr 15 cct Pro | Pro ccc Pro | |
| <22 <40 atg Met 1 tat Tyr ttt Phe | 1> CI 2> (: 0> 1: aag Lys gac Asp | OS 1) 1 cgc Arg acg Thr tcc Ser 35 | gca Ala gaa Glu 20 ccc Pro | aga Arg 5 acc Thr aat Asn | ggt Gly gga Gly cct | cct Pro | Glu cca Pro caa Gln 40 | act Thr 25 gag Glu acc | Thr 10 gtg Val agt ser | Phe cct Pro ccc Pro | Asn ttt Phe cct Pro | Pro ctt Leu ggg Gly 45 atg | act Thr 30 gta Val | Tyr 15 cct Pro ctc Leu | Pro ccc Pro tct Ser ctc | 96 |
| <22: <40 atg Met 1 tat Tyr ttt Phe ttg Leu aaa | 1> CI 2> (: 0> 1: aag Lys gac Asp gta Val cgc Arg | os 1) 1 cgc Arg acg Thr tcc ser 35 cta Leu | gca Ala gaa Glu 20 ccc Pro tcc Ser | aga Arg 5 acc Thr aat Asn gaa Glu | ggt Gly ggg Gly cct Pro | cct Pro ttt Phe cta Leu 55 | CCa Pro Caa Gln 40 gtt Val | act Thr 25 gag Glu acc Thr | Thr 10 gtg Val agt ser tcc ser gag | Phe cct Pro ccc Pro aat Asn | Asn ttt Phe cct Pro ggc Gly 60 ggc | Pro ctt Leu ggg Gly 45 atg Met aac | act Thr 30 gta Val ctt Leu | Tyr 15 cct Pro ctc Leu gcg Ala | Pro ccc Pro tct Ser ctc Leu | 96 144 |

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| ata Ile | aac Asn | ctg Leu | gaa Glu 100 | ata Ile | tct Ser | gca Ala | ccc Pro | ctc Leu 105 | aca Thr | gtt Val | acc Thr | tca Ser | gaa Glu 110 | gcc Ala | cta Leu | 336 |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| act Thr | gtg Val | gct Ala 115 | gcc Ala | gcc Ala | gca Ala | cct Pro | cta Leu 120 | atg Met | gtc Val | gcg Ala | ggc Gly | aac Asn 125 | aca Thr | ctc Leu | acc Thr | 384 |
| | caa Gln 130 | | | | | | | | | | | | | | | 432 |
| gcc Ala 145 | acc Thr | caa Gln | gga Gly | ccc Pro | ctc Leu 150 | aca Thr | gtg Val | tca Ser | gaa Glu | gga Gly 155 | aag Lys | cta Leu | gcc Ala | ctg Leu | caa Gln 160 | 480 |
| aca Thr | tca Ser | ggc | ccc Pro | ctc Leu 165 | acc Thr | acc Thr | acc Thr | gat Asp | agc Ser 170 | agt Ser | acc Thr | ctt Leu | act Thr | atc Ile 175 | act Thr | 528 |
| gcc Ala | tca Ser | ccc Pro | cct Pro 180 | cta Leu | act Thr | act Thr | gcc Ala | act Thr 185 | ggt Gly | agc Ser | ttg Leu | ggc Gly | att Ile 190 | gac Asp | ttg Leu | 576 |
| | gag Glu | | | | | | | | | | | | | | | 624 |
| gct Ala | cct Pro 210 | ttg Leu | cat His | gta Val | aca Thr | gac Asp 215 | gac Asp | cta Leu | aac Asn | act Thr | ttg Leu 220 | acc Thr | gta Val | gca Ala | act Thr | 672 |
| | cca Pro | | | | | | | | | | | | | | | 720 |
| | gcc Ala | | | | | | | | | | | | | | | 768 |
| | gga Gly | | | | _ | | | | _ | _ | | | | _ | _ | 816 |
| | tat Tyr | | | | | | | | | | | | | | cag Gln | 864 |
| | cct Pro 290 | | | | | | | | | | | | | | | 912 |
| | gly ggc | | | | | | | | | | | | | | | 960 |
| | aac Asn | | | | | | | | | | | | | | | 1008 |

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| gcc Ala | att Ile | aat Asn | gca Ala 340 | gga Gly | gat Asp | gl ^à aaa | ctt Leu | gaa Glu 345 | ttt Phe | ggt Gly | tca Ser | cct Pro | aat Asn 350 | gca Ala | cca Pro | 1056 |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| aac Asn | aca Thr | aat Asn 355 | ccc Pro | ctc Leu | aaa Lys | aca Thr | aaa Lys 360 | att Ile | ggc Gly | cat His | ggc | cta Leu 365 | gaa Glu | ttt Phe | gat Asp | 1104 |
| tca Ser | aac Asn 370 | aag Lys | gct Ala | atg Met | gtt Val | cct Pro 375 | aaa Lys | cta Leu | gga Gly | act Thr | ggc 380 | ctt Leu | agt Ser | ttt Phe | gac Asp | 1152 |
| agc Ser 385 | aca Thr | ggt Gly | gcc Ala | att Ile | aca Thr 390 | gta Val | gga Gly | aac Asn | aaa Lys | aat Asn 395 | aat Asn | gat Asp | aag Lys | cta Leu | act Thr 400 | 1200 |
| ttg Leu | tgg Trp | acc Thr | aca Thr | cca Pro 405 | gct Ala | cca Pro | tct Ser | cct Pro | aac Asn 410 | tgt Cys | aga Arg | cta Leu | aat Asn | gca Ala 415 | gag Glu | 1248 |
| aaa Lys | gat Asp | gct Ala | aaa Lys 420 | ctc Leu | act Thr | ttg Leu | gtc Val | tta Leu 425 | aca Thr | aaa Lys | tgt Cys | ggc Gly | agt Ser 430 | caa Gln | ata Ile | 1296 |
| ctt Leu | gct Ala | aca Thr 435 | gtt Val | tca Ser | gtt Val | ttg Leu | gct Ala 440 | gtt Val | aaa Lys | ggc Gly | agt Ser | ttg Leu 445 | gct Ala | cca Pro | ata Ile | 1344 |
| tct Ser | gga Gly 450 | aca Thr | gtt Val | caa Gln | agt Ser | gct Ala 455 | cat His | ctt Leu | att Ile | ata Ile | aga Arg 460 | ttt Phe | gac Asp | gaa Glu | aat Asn | 1392 |
| gga Gly 465 | gtg Val | cta Leu | cta Leu | aac Asn | aat Asn 470 | tcc Ser | ttc Phe | ctg Leu | gac Asp | cca Pro 475 | gaa Glu | tat Tyr | tgg Trp | aac Asn | ttt Phe 480 | 1440 |
| aga Arg | aat Asn | gga Gly | gat Asp | ctt Leu 485 | act Thr | gaa Glu | ggc Gly | aca Thr | gcc Ala 490 | tat Tyr | aca Thr | aac Asn | gct Ala | gtt Val 495 | gga Gly | 1488 |
| ttt Phe | atg Met | cct Pro | aac Asn 500 | cta Leu | tca Ser | gct Ala | tat Tyr | cca Pro 505 | aaa Lys | tct Ser | cac His | ggt Gly | aaa Lys 510 | act Thr | gcc Ala | 1536 |
| aaa Lys | agt Ser | aac Asn 515 | att Ile | gtc Val | Ser | caa Gln | Val | Tyr | tta Leu | aac Asn | gga Gly | gac Asp 525 | aaa Lys | act Thr | aaa Lys | 1584 |
| cct Pro | gta Val 530 | aca Thr | cta Leu | acc Thr | att Ile | aca Thr 535 | cta Leu | aac Asn | ggt Gly | aca Thr | cag Gln 540 | gaa Glu | aca Thr | ggt Gly | gat Asp | 1632 |
| cat His 545 | tgt Cys | gat Asp | tgt Cys | cgt Arg | ggt Gly 550 | gat Asp | tgt Cys | ttt Phe | tgt Cys | aca Thr 555 | act Thr | cca Pro | agt Ser | gca Ala | tac Tyr 560 | 1680 |
| tct Ser | atg Met | tca Ser | ttt Phe | tca Ser 565 | tgg Trp | gac Asp | tgg Trp | tct Ser | ggc Gly 570 | cac His | aac Asn | tac Tyr | att Ile | aat Asn 575 | gaa Glu | 1728 |
| ata | ttt | gcc | aca | tcc | tct | taca | cttt | tt c | atac | attg | la ac | aaga | ataa. | L | | 1776 |

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Ile Phe Ala Thr Ser Ser 580

<210> 12 <211> 582 <212> PRT <213> Artificial Sequence <220> <223> 5F S*RGD <400> 12 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro 5 10 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro 2.0 25 3.0 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser 35 40 45 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu 55 60 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser 70 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn 90 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu 105 100 110 Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr 115 120 125 Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile 135 140 Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln 150 155 Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr 170 165 Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu 180 185 190 Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly 200 Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr 220 215 Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr 230 235 Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala 250 245 Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val 260 265 270 Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln 275 280 285 Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn 295 300 Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu 310 315 Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile 325 330 335 Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro 345 350 Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp 360 Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp

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| | 370 | | | | | 375 | | | | | 380 | | | | | |
|------------------------------|---------------------|--------------------|------------------|-----------------|-----------------|------------------|------------------|------------------|------------------|------------|------------------|------------------|------------------|------------------|------------|-----|
| Ser | Thr | Gly | Ala | Ile | | Val | ${	t Gly}$ | Asn | Lys | | Asn | Asp | Lys | Leu | Thr | |
| 385 Leu | Trp | Thr | Thr | | 390 Ala | Pro | Ser | Pro | | 395 Cys | Arg | Leu | Asn | Ala | 400 Glu | |
| Lys | Asp | Ala | | 405 Leu | Thr | Leu | Val | Leu 425 | 410 Thr | Lys | Cys | Gly | Ser 430 | 415 Gln | Ile | |
| Leu | Ala | Thr 435 | 420 Val | Ser | Val | Leu | Ala 440 | | Lys | Gly | Ser | Leu 445 | | Pro | Ile | |
| Ser | Gly 450 | | Val | Gln | Ser | Ala 455 | | Leu | Ile | Ile | Arg 460 | | Asp | Glu | Asn | |
| Gly 465 | | Leu | Leu | Asn | Asn 470 | | Phe | Leu | Asp | Pro 475 | Glu | Тух | Trp | Asn | Phe 480 | |
| | | | | 485 | | | | | 490 | | | | | Val 495 | | |
| | | | 500 | | | | | 505 | | | | | 510 | Thr | | |
| _ | | 515 | | | | | 520 | | | | | 525 | | Thr | | |
| | 530 | | | | | 535 | | | | | 540 | | | Gly | | |
| 545 | | | | | 550 | | | | | 555 | | | | Ala | 560 | |
| | | | | 565 | | Asp | Trp | Ser | Gly 570 | His | Asn | Tyr | Ile | Asn 575 | GIu | |
| Ile | Phe | Ala | Thr 580 | Ser | Ser | | | | | | | | | | | |
| <21: <21: <21: <22: | | 746 NA rtifi | | l Sed | queno | ce | | | | | | | | | | |
| | 3 > 5: | | LS* | | | | | | | | | • | | | | |
| | 1> C 2> (| | . (17 | 46) | | | | | | | | | | | | |
| atq | 0> 1. aag Lys | cqc | gca Ala | aga Arg 5 | ccg Pro | tct Ser | gaa Glu | gat Asp | acc Thr 10 | ttc Phe | aac Asn | ccc Pro | gtg Val | tat Tyr 15 | cca Pro | 48 |
| tat Tyr | gac Asp | acg Thr | gaa Glu 20 | acc Thr | ggt Gly | cct Pro | cca Pro | act Thr 25 | gtg Val | cct Pro | ttt Phe | ctt Leu | act Thr 30 | cct Pro | ccc Pro | 96 |
| ttt Phe | gta Val | tcc Ser 35 | ccc Pro | aat Asn | 61 ³ | ttt Phe | caa Gln 40 | gag Glu | agt Ser | ccc Pro | cct Pro | 999 Gly 45 | gta Val | ctc Leu | tct Ser | 144 |
| ttg Leu | cgc Arg 50 | cta Leu | tcc Ser | gaa Glu | cct Pro | cta Leu 55 | gtt Val | acc Thr | tcc Ser | aat Asn | ggc Gly 60 | atg Met | ctt Leu | gcg Ala | ctc Leu | 192 |
| aaa Lys | ato | aac | aac | ggc | ctc | tct | ctg | gac | gag | gcc | ggc | aac | ctt | acc | tcc | 240 |

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| caa Gln | aat Asn | gta Val | acc Thr | act Thr 85 | gtg Val | agc Ser | cca Pro | cct Pro | ctc Leu 90 | gga Gly | gcc Ala | gga Gly | gcc Ala | tca Ser 95 | aac Asn | 288 |
|------------|------------|------------|------------|------------------|------------|------------|------------|------------|------------------|------------|------------|------------|-------------------|------------------|------------|------|
| | | | | | | | | | | | | | gaa Glu 110 | | | 336 |
| | | | | | | | | | | | | | aca Thr | | | 384 |
| | | | | | | | | | | | | | ctt Leu | | | 432 |
| | | | | | | | | | | | | | gcc Ala | | | 480 |
| | | | | | | | | | | | | | act Thr | | | 528 |
| | | | | | | | | | | | | | att Ile 190 | | | 576 |
| | | | | | | | | | | | | | aag Lys | | | 624 |
| | | | | | | | | | | | | | gta Val | | | 672 |
| | | | | | | | | | | | | | aaa Lys | | | 720 |
| | | | | | | | | | | | | | aat Asn | | | 768 |
| | | | Arg | Ile | Āsp | | Gln | Asn | Ārg | | | | ctt Leu 270 | | | 816 |
| | | | | | | | | | | | | | cta Leu | | | 864 |
| | | | | | | | | | | | | | aac Asn | | | 912 |
| | | | | | | | | | | | | | aag Lys | | | 960 |
| gtt | aac | cta | agc | act | gcc | aag | aaa | ttg | atg | ttt | gac | gct | aca | gcc | ata | 1008 |

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| Val | Asn | Leu | Ser | Thr 325 | Ala | Lys | Gly | Leu | Met 330 | Phe | Asp | Ala | Thr | Ala 335 | Ile | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------|-------------------|-------------------|-------------------|-------------------|------|
| gcc Ala | att Ile | aat Asn | gca Ala 340 | Gly | gat Asp | gjà aaa | ctt Leu | gaa Glu 345 | ttt Phe | ggt Gly | tca Ser | cct Pro | aat Asn 350 | gca Ala | cca Pro | 1056 |
| aac Asn | aca Thr | aat Asn 355 | ccc Pro | ctc Leu | aaa Lys | aca Thr | aaa Lys 360 | att Ile | gly ggc | cat His | ggc | cta Leu 365 | gaa Glu | ttt Phe | gat Asp | 1104 |
| tca Ser | aac Asn 370 | aag Lys | gct Ala | atg Met | gtt Val | cct Pro 375 | aaa Lys | cta Leu | gga Gly | act Thr | ggc 380 | ctt Leu | agt Ser | ttt Phe | gac Asp | 1152 |
| agc Ser 385 | aca Thr | ggt Gly | gcc Ala | att Ile | aca Thr 390 | gta Val | gga Gly | aac Asn | aaa Lys | aat Asn 395 | aat Asn | gat Asp | aag Lys | cta Leu | act Thr 400 | 1200 |
| ttg Leu | tgg Trp | acc Thr | aca Thr | cca Pro 405 | gct Ala | cca Pro | gag Glu | gct Ala | aac Asn 410 | tgt Cys | aga Arg | cta Leu | aat Asn | gca Ala 415 | gag Glu | 1248 |
| | gat Asp | | | | | | | | | | | | | | | 1296 |
| ctt Leu | gct Ala | aca Thr 435 | gtt Val | tca Ser | gtt Val | ttg Leu | gct Ala 440 | gtt Val | aaa Lys | ggc Gly | agt Ser | ttg Leu 445 | gct Ala | cca Pro | ata Ile | 1344 |
| | gga Gly 450 | | | | | | | | | | | | | | | 1392 |
| | gtg Val | | | | | | | | | | | | | | | 1440 |
| aga Arg | aat Asn | gga Gly | gat Asp | ctt Leu 485 | act Thr | gaa Glu | ggc | aca Thr | gcc Ala 490 | tat Tyr | aca Thr | aac Asn | gct Ala | gtt Val 495 | gga Gly | 1488 |
| ttt Phe | atg Met | cct Pro | aac Asn 500 | cta Leu | tca Ser | gct Ala | tat Tyr | cca Pro 505 | aaa Lys | tct Ser | cac His | ggt Gly | aaa Lys 510 | act Thr | gcc Ala | 1536 |
| | agt Ser | | | | | | | | | | | | | | | 1584 |
| | gta Val 530 | | | | | | | | | | | | | | | 1632 |
| | act Thr | | | | | | | | | | | | | | | 1680 |
| | aac Asn | | | | | | | | | | | | | | | 1728 |

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tac att gcc caa gaa taa Tyr Ile Ala Gln Glu * <210> 14 <211> 581 <212> PRT <213> Artificial Sequence <220> <223> 5F KO1S* <400> 14 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser Gln Asn Val Thr Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu Thr Val Ala Ala Ala Aro Leu Met Val Ala Gly Asn Thr Leu Thr Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu 305 310 315 320
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp

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```
360
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
                      375
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
                    390
                                        395
Leu Trp Thr Thr Pro Ala Pro Glu Ala Asn Cys Arg Leu Asn Ala Glu
                                    410
               405
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
            420
                                425
                                                     430
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
        435
                            440
                                                 445
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
            455
                                            460
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
                    470
                                         475
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
                485
                                    490
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
           500
                                505
                                                     51.0
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
                            520
        515
                                                 525
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
                        535
                                            540
Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
                            , 555
                    550
His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
Tyr Ile Ala Gln Glu
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                                                                    48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act ccc Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
             20
ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct
                                                                    144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
                              40
ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc
                                                                    192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
                         55
                                             60
aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
```

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| 65 | | | | | 70 | | | | | 75 | | | | | 80 | |
|-------------------|------------|-------------------|------------|------------|-------------------|------------|-------------------|------------|------------|-------------------|-------------------|-------------------|------------|------------|-------------------|-----|
| | | | | | | | | | | | gcc Ala | | | | | 288 |
| | | _ | _ | | | _ | | | | _ | acc Thr | | _ | _ | | 336 |
| | | | | | | | | | | | ggc | | | | | 384 |
| | | | | | | | | | | | tcc Ser 140 | | | | | 432 |
| | | | | | | | | | | | aag Lys | | | | | 480 |
| | | | | | | | | | | | acc Thr | | | | | 528 |
| | | | | | | | | | | | ttg Leu | | | | | 576 |
| | | | | | | | | | | | gga Gly | | | | | 624 |
| | | | | | | | | | | | ttg Leu 220 | | | | | 672 |
| | | | | | | | | | | | caa Gln | | | | | 720 |
| | | | | | | | | | | | caa Gln | | | | | 768 |
| | | | | | | | | | | | ctt Leu | | | | | 816 |
| agt Ser | tat Tyr | ccg Pro 275 | ttt Phe | gat Asp | gct Ala | caa Gln | aac Asn 280 | caa Gln | cta Leu | aat Asn | cta Leu | aga Arg 285 | cta Leu | gga Gly | cag Gln | 864 |
| | | | | | | | | | | | gat Asp 300 | | | | | 912 |
| aaa Lys 305 | ggc Gly | ctt Leu | tac Tyr | ttg Leu | ttt Phe 310 | aca Thr | gct Ala | tca Ser | aac Asn | aat Asn 315 | tcc Ser | aaa Lys | aag Lys | ctt Leu | gag Glu 320 | 960 |

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| | | | | | gcc Ala | | | | | | | | | | | 1008 |
|------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|------|
| gcc Ala | att Ile | aat Asn | gca Ala 340 | gga Gly | gat Asp | gly aaa | ctt Leu | gaa Glu 345 | ttt Phe | ggt Gly | tca Ser | cct Pro | aat Asn 350 | gca Ala | cca Pro | 1056 |
| | | | | | aaa Lys | | | | | | | | | | | 1104 |
| tca Ser | aac Asn 370 | aag Lys | gct Ala | atg Met | gtt Val | cct Pro 375 | aaa Lys | cta Leu | gga Gly | act Thr | ggc Gly 380 | ctt Leu | agt Ser | ttt Phe | gac Asp | 1152 |
| | | | | | aca Thr 390 | | | | | | | | | | | 1200 |
| ttg Leu | tgg Trp | acc Thr | aca Thr | cca Pro 405 | gct Ala | cca Pro | gag Glu | gct Ala | aac Asn 410 | tgt Cys | aga Arg | cta Leu | aat Asn | gca Ala 415 | gag Glu | 1248 |
| | | | | | act Thr | | | | | | | | | | | 1296 |
| ctt Leu | gct Ala | aca Thr 435 | gtt Val | tca Ser | gtt Val | ttg Leu | gct Ala 440 | gtt Val | aaa Lys | ggc Gly | agt Ser | ttg Leu 445 | gct Ala | cca Pro | ata Ile | 1344 |
| tct Ser | gga Gly 450 | aca Thr | gtt Val | caa Gln | agt Ser | gct Ala 455 | cat His | ctt Leu | att Ile | ata Ile | aga Arg 460 | ttt Phe | gac Asp | gaa Glu | aat Asn | 1392 |
| | | | | | aat Asn 470 | | | | | | | | | | | 1440 |
| | | | | | act Thr | | | | | | | | | | | 1488 |
| | | | | | tca Ser | | Tyr | Pro | | Ser | | | | Thr | | 1536 |
| aaa Lys | agt Ser | aac Asn 515 | att Ile | gtc Val | agt Ser | caa Gln | gtt Val 520 | tac Tyr | tta Leu | aac Asn | gga Gly | gac Asp 525 | aaa Lys | act Thr | aaa Lys | 1584 |
| | | | | | att Ile | | | | | | | | | | | 1632 |
| | Cys | | | | ggt Gly 550 | | | | | | | | | | | 1680 |

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tct atg tca ttt tca tgg gac tgg tct ggc cac aac tac att aat gaa Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu 1728 565 ata ttt gcc aca tcc tct tac act ttt tca tac att gcc caa gaa taa 1776 Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile Ala Gln Glu * 585 580 <210> 16 <211> 591 <212> PRT <213> Artificial Sequence <223> 5F KO1S*RGD <400> 16 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro 10 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro 20 25 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser 35 40 45 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu 55 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser 70 75 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn 85 90 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu 105 Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr 120 125 Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile 135 140 Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln 150 155 Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr 170 165 Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu 180 185 Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly 200 Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr 215 220 Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr 230 235 Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala 250 245 Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val 260 265 Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln 280 Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn 295 300 Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu 310 315 320 Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile

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Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro

```
340
                               345
                                               350
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
                           360
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
                        375
                                            380
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
                   390
                                       395
Leu Trp Thr Thr Pro Ala Pro Glu Ala Asn Cys Arg Leu Asn Ala Glu
               405
                                   410
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
                               425
                                                  430
          420
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
                          440
                                               445
       435
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
   450
                       455
                                            460
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
                  470
                                       475
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
               485
                                   490
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
                               505
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
                                               525
       515
                           520
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
                       535
                                           540
His Cys Asp Cys Arg Gly Asp Cys Phe Cys Thr Thr Pro Ser Ala Tyr
                   550
                                       555
Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu
               565
                                   570
Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile Ala Gln Glu
<210> 17
<211> 972
<212> DNA
<213> Artificial Sequence
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<223> Ad35 fiber
<221> CDS
<222> (1)...(972)
atg acc aag aga gtc cgg ctc agt gac tcc ttc aac cct gtc tac ccc
                                                                  48
Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
                                    10
tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt
Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta
Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa
Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys
```

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| | gga Gly | | | | | | | | | | | | | | | 240 |
|------------|-------------------|-------------------|-------------------|-------------------|------------------------|-------------------|-------------------|-------------------|-------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|-----|
| aac Asn | ata Ile | cgt Arg | gct Ala | aca Thr 85 | gca Ala | ccc Pro | att Ile | act Thr | aaa Lys 90 | aat Asn | aat Asn | cac His | tct Ser | gta Val 95 | gaa Glu | 288 |
| cta Leu | tcc Ser | att Ile | gga Gly 100 | aat Asn | gga Gly | tta Leu | gaa Glu | act Thr 105 | caa Gln | aac Asn | aat Asn | aaa Lys | cta Leu 110 | tgt Cys | gcc Ala | 336 |
| | ttg Leu | | | | | | | | | | | | | | | 384 |
| | agt Ser 130 | | | | | | | | | | | | | | | 432 |
| | att Ile | | | | | | | | | | | | | | | 480 |
| tta Leu | gta Val | aaa Lys | aat Asn | gga Gly 165 | gl ^à aaa | ctt Leu | gtt Val | aat Asn | ggc Gly 170 | tac Tyr | gtg Val | tct Ser | cta Leu | gtt Val 175 | ggt Gly | 528 |
| gta Val | tca Ser | gac Asp | act Thr 180 | gtg Val | aac Asn | caa Gln | atg Met | ttc Phe 185 | aca Thr | caa Gln | aag Lys | aca Thr | gca Ala 190 | aac Asn | atc Ile | 576 |
| caa Gln | tta Leu | aga Arg 195 | tta Leu | tat Tyr | ttt Phe | gac Asp | tct Ser 200 | tct Ser | gga Gly | aat Asn | cta Leu | tta Leu 205 | act Thr | gag Glu | gaa Glu | 624 |
| tca Ser | gac Asp 210 | tta Leu | aaa Lys | att Ile | cca Pro | ctt Leu 215 | aaa Lys | aat Asn | aaa Lys | tct Ser | tct Ser 220 | aca Thr | gcg Ala | acc Thr | agt Ser | 672 |
| | act Thr | | | | | | | | | | | | | | | 720 |
| ccc Pro | ttc Phe | aac Asn | acc Thr | act Thr 245 | act Thr | agg Arg | gat Asp | agt Ser | gaa Glu 250 | aac Asn | tac Tyr | att Ile | cat His | gga Gly 255 | ata Ile | 768 |
| | tac Tyr | | | | | | | | | | | | | | | 816 |
| tct Ser | ata Ile | atg Met 275 | cta Leu | aac Asn | agc Ser | cgt Arg | atg Met 280 | att Ile | tct Ser | tcc Ser | aat Asn | gtt Val 285 | gcc Ala | tat Tyr | gcc Ala | 864 |
| ata Ile | caa Gln 290 | ttt Phe | gaa Glu | tgg Trp | aat Asn | cta Leu 295 | aat Asn | gca Ala | agt Ser | gaa Glu | tct Ser 300 | cca Pro | gaa Glu | agc Ser | aac Asn | 912 |

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ata gct acg ctg acc aca tcc ccc ttt ttc ttt tct tac att aca gaa 960 Ile Ala Thr Leu Thr Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu 310 972 gac gac gaa taa Asp Asp Glu * <210> 18 <211> 323 <212> PRT <213> Artificial Sequence <220> <223> Ad 35 fiber <400> 18 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro 10 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe 20 25 Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu 40 45 35 Lys Cys Leu Thr Pro Leu Thr Thr Thr Gly Gly Ser Leu Gln Leu Lys 55 50 Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu 75 70 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu 85 90 Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala 105 110 100 Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys 120 115 Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys 135 140 Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val 155 150 Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly 165 170 Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile 180 185 Gln Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu 195 200 205 Ser Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser 215 220 Glu Thr Val Ala Ser Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr 230 235 240 Pro Phe Asn Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile 250 245 Cys Tyr Tyr Met Thr Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile 265 260 Ser Ile Met Leu Asn Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala 280 285 275 Ile Gln Phe Glu Trp Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn 295 300 Ile Ala Thr Leu Thr Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu 305 310 315 Asp Asp Glu

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<210> 19
<211> 1002
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<213> Artificial Sequence
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<223> 35F RGD
<221> CDS
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Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt
Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta
                                                                           144
Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
                                 40
                                                                           192
aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa
Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys
gtg gga ggg gga ctt aca gtg gat gac act gat ggt acc tta caa gaa Val Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
                                                                           240
                       70
aac ata cgt gct aca gca ccc att act aaa aat aat cac tct gta gaa
                                                                           288
Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
                                          90
cta tcc att gga aat gga tta gaa act caa aac aat aaa cta tgt gcc
                                                                           336
Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala
aaa ttg gga aat ggg tta aaa ttt aac aac ggt gac att tgt ata aag
Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
gat agt att aac acc tta tgg act gga ata aac cct cca cct aac tgt
                                                                           432
Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys
                           135
caa att gtg gaa aac act aat aca aat gat ggc aaa ctt act tta gta
                                                                           480
Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val
tta gta aaa aat gga ggg ctt gtt aat ggc tac gtg tct cta gtt ggt
Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly
gta tca gac act gtg aac caa atg ttc aca caa aag aca gca aac atc Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile
             180
                                                           190
                                    185
caa tta aga tta tat ttt gac tct tct gga aat cta tta act gag gaa
```

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| | u Arg 195 | Leu | Tyr | Phe | Asp | Ser 200 | Ser | Gly | Asn | Leu | Leu 205 | Thr | Glu | Glu | |
|--|---|---------------------------------------|--------------------------------------|--|---------------------------------------|--------------------------------|--------------------------------|--|---------------------------------------|--------------------------------|--------------------------------|---------------------------------------|--|--------------------------------|------|
| tca ga Ser Asj 21 | p Leu | aaa Lys | att Ile | cca Pro | ctt Leu 215 | aaa Lys | aat Asn | aaa Lys | tct Ser | tct Ser 220 | aca Thr | gcg Ala | acc Thr | agt Ser | 672 |
| gaa ac Glu Th 225 | t gta r Val | gcc Ala | agc Ser | agc Ser 230 | aaa Lys | gcc Ala | ttt Phe | atg Met | cca Pro 235 | agt Ser | act Thr | aca Thr | gct Ala | tat Tyr 240 | 720 |
| ccc tt Pro Ph | c aac e Asn | acc Thr | act Thr 245 | act Thr | agg Arg | gat Asp | agt Ser | gaa Glu 250 | aac Asn | tac Tyr | att Ile | cat His | gga Gly 255 | ata Ile | 768 |
| tgt ta Cys Ty | c tac r Tyr | atg Met 260 | act Thr | agt Ser | tat Tyr | gat Asp | aga Arg 265 | agt Ser | cta Leu | ttt Phe | ccc Pro | ttg Leu 270 | aac Asn | att Ile | 816 |
| tct at Ser Il | a atg e Met 275 | cta Leu | aac Asn | agc Ser | cgt Arg | atg Met 280 | att Ile | tct Ser | tcc Ser | aat Asn | gta Val 285 | cat His | tgt Cys | gat Asp | 864 |
| tgt cg Cys Ar 29 | g Gly | gat Asp | tgt Cys | ttt Phe | tgc Cys 295 | gca Ala | tat Tyr | gcc Ala | ata Ile | caa Gln 300 | ttt Phe | gaa Glu | tgg Trp | aat Asn | 912 |
| cta aa Leu As 305 | it gca sn Ala | agt Ser | gaa Glu | tct Ser 310 | cca Pro | gaa Glu | agc Ser | aac Asn | ata Ile 315 | gct Ala | acg Thr | ctg Leu | acc Thr | aca Thr 320 | 960 |
| tcc cc Ser Pr | cc ttt co Phe | ttc Phe | ttt Phe 325 | tct Ser | tac Tyr | att Ile | aca Thr | gaa Glu 330 | gac Asp | gac Asp | gaa Glu | taa * | | | 1002 |
| | | | | | | | | | | | | | | | |
| <210><211><211><212><213> | 332 PRT | icia | l Sed | quen | ce | | | | | | | | | | |
| <211> <212> | 332 PRT Artif | | l Sed | quen | ce | | | | | | | | | | |
| <211> <212> <213> | 332 PRT Artif | D | Arg | _ | | Asp | Ser | | Asn | Pro | Val | Tyr | Pro | Tyr | |
| <211><212><213><213> 10 10 | 332 PRT Artif 35FRG 20 ys Arg | D Val Ser | Arg 5 | Leu | Ser | | Pro | 10 | | | | | 15 | | |
| <211> <212> <213> <220> <223> <400> Thr Ly | 332 PRT Artif 35FRG 20 Vs Arg sp Glu | D Val Ser 20 | Arg 5 Thr | Leu Ser | Ser Gln | His Ser | Pro 25 | 10 Phe | Ile | Asn | Pro Leu | Gly 30 | 15 Phe | Ile | |
| <211> <212> <213> <220> <223> <400> Thr Ly 1 Glu As Ser Pr Cys Le | 332 PRT Artif 35FRG 20 ys Arg sp Glu ro Asn 35 eu Thr | Val Ser 20 Gly | Arg 5 Thr | Leu Ser Thr | Ser Gln Gln Thr | His Ser 40 | Pro 25 Pro | 10 Phe Asp | Ile Gly | Asn Val Leu | Pro Leu 45 | Gly 30 Thr | 15 Phe Leu | Ile Lys | |
| <211> <212> <213> <220> <223> <400> Thr Ly 1 Glu As Ser Pr Cys Le 50 Gly Gl | 332 PRT Artif 35FRG 20 ys Arg sp Glu ro Asn 35 au Thr | Val Ser 20 Gly Pro | Arg 5 Thr Phe Leu | Leu Ser Thr Thr | Ser Gln Gln Thr | His Ser 40 Thr | Pro 25 Pro Gly | 10 Phe Asp Gly | Ile Gly Ser Gly | Asn Val Leu 60 | Pro Leu 45 Gln | Gly 30 Thr | 15 Phe Leu Lys | Ile Lys Val Asn | |
| <211> <212> <213> <220> <223> <400> Thr Ly 1 Glu As Ser Pr Cys Le | 332 PRT Artif 35FRG 20 ys Arg sp Glu ro Asn 35 eu Thr 0 | Val Ser 20 Gly Pro | Arg 5 Thr Phe Leu Thr | Leu Ser Thr Thr Val | Ser Gln Gln Thr 55 Asp | His Ser 40 Thr Asp | Pro 25 Pro Gly Thr | 10 Phe Asp Gly Asp | Ile Gly Ser Gly 75 | Asn Val Leu 60 Thr | Pro Leu 45 Gln Leu | Gly 30 Thr Leu Gln | 15 Phe Leu Lys Glu Glu | Ile Lys Val Asn 80 | |
| <pre><211> <212> <213> <220> <223> <400> Thr Ly 1 Glu As Ser Pr Cys Le 50 Gly Gl 65</pre> | 332 PRT Artif 35FRG 20 ys Arg sp Glu ro Asn 35 eu Thr Oly Gly | Val Ser 20 Gly Pro Leu | Arg 5 Thr Phe Leu Thr Ala 85 Gly | Leu Ser Thr Thr Val 70 Pro | Ser Gln Gln Thr 55 Asp | His Ser 40 Thr Asp | Pro 25 Pro Gly Thr | 10 Phe Asp Gly Asp Asn 90 Asn | Ile Gly Ser Gly 75 Asn | Asn Val Leu 60 Thr | Pro Leu 45 Gln Leu Ser | Gly 30 Thr Leu Gln Val | 15 Phe Leu Lys Glu Glu 95 Ala | Lys Val Asn 80 Leu | |

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| 715 | | | 100 | | | | | 100 | | | | |
|---|--------------------------|----------------|----------------------|------------------|------------|------------|------------|------------|------------------|------------|------------|-----|
| Ser Ile Asn 130 | Thr Leu | | 120 hr Gly 135 | | Asn | Pro | Pro 140 | 125 Pro | Asn | Cys | Gln | |
| Ile Val Glu 145 | Asn Thr | | | . Asp | Gly | Lys 155 | | Thr | Leu | Val | Leu 160 | |
| Val Lys Asn | Gly Gly 165 | | /al Asn | Gly | Tyr 170 | Val | Ser | Leu | Val | Gly 175 | | |
| Ser Asp Thr | Val Asn 180 | Gln M | Met Phe | Thr 185 | | Lys | Thr | Ala | Asn 190 | | Gln | |
| Leu Arg Leu 195 | Tyr Phe | Asp S | Ser Ser 200 | _ | Asn | Leu | Leu | Thr 205 | Glu | Glu | Ser | |
| Asp Leu Lys 210 | Ile Pro | | iys Asn 215 | Lys | Ser | Ser | Thr 220 | Ala | Thr | Ser | Glu | |
| Thr Val Ala 225 | Ser Ser | Lys A 230 | Ala Phe | Met | Pro | Ser 235 | | Thr | Ala | Tyr | Pro 240 | |
| Phe Asn Thr | Thr Thr 245 | Arg A | Asp Ser | Glu | Asn 250 | Tyr | Ile | His | Gly | Ile 255 | | |
| Tyr Tyr Met | Thr Ser 260 | Tyr A | Asp Arg | Ser 265 | | Phe | Pro | Leu | Asn 270 | | Ser | |
| Ile Met Leu 275 | Asn Ser | Arg M | Met Ile 280 | | Ser | Asn | Val | His 285 | Cys | Asp | Cys | |
| Arg Gly Asp 290 | Cys Phe | | Ala Tyr 295 | Ala | Ile | Gln | Phe 300 | Glu | Trp | Asn | Leu | |
| Asn Ala Ser 305 | Glu Ser | Pro G 310 | Hu Ser | Asn | Ile | Ala 315 | Thr | Leu | Thr | Thr | Ser 320 | |
| Pro Phe Phe | Phe Ser 325 | Tyr I | le Thr | Glu | Asp 330 | | Glu | | | | | |
| <210> 21 <211> 1164 <212> DNA <213> Artifi | cial Sec | quence | : | | | | | | | | | |
| <220> <223> Ad41 s | short fil | ber | | | | | | | | | | |
| <221> CDS <222> (1) | (1164) | | | | | | | | | | | |
| <400> 21 | | | | | | | | | | | | |
| atg aaa aga Met Lys Arg 1 | | | | | | | | | | | | 48 |
| gac acc ttc Asp Thr Phe | tca act Ser Thr 20 | ccc a Pro S | gc atc Ser Ile | ccc Pro 25 | tat Tyr | gta Val | gct Ala | ccg Pro | ccc Pro 30 | ttc Phe | gtt Val | 96 |
| tct tct gac Ser Ser Asp 35 | | | | | | | | | | | | 144 |
| tac act gac Tyr Thr Asp 50 | | Thr T | | | | | | | | | | 192 |
| | | | | | | | | | | | | |

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| ccc Pro | act Thr | gtt Val | tct Ser | cct Pro 85 | ccc Pro | ctt Leu | aca Thr | aac Asn | agt Ser 90 | aac Asn | aac Asn | tcc Ser | ctg Leu | ggt Gly 95 | tta Leu | 288 |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| gcc Ala | aca Thr | tcc Ser | gct Ala 100 | ccc Pro | ata Ile | gct Ala | gta Val | tca Ser 105 | gct Ala | aac Asn | tct Ser | ctc Leu | aca Thr 110 | ttg Leu | gcc Ala | 336 |
| acc Thr | gcc Ala | gca Ala 115 | cca Pro | ctg Leu | aca Thr | gta Val | agc Ser 120 | aac Asn | aac Asn | cag Gln | ctt Leu | agt Ser 125 | att Ile | aac Asn | gcg Ala | 384 |
| gly ggc | aga Arg 130 | ggt Gly | tta Leu | gtt Val | ata Ile | act Thr 135 | aac Asn | aat Asn | gcc Ala | tta Leu | aca Thr 140 | gtt Val | aat Asn | cct Pro | acc Thr | 432 |
| gga Gly 145 | gcg Ala | cta Leu | ggt Gly | ttc Phe | aat Asn 150 | aac Asn | aca Thr | gga Gly | gct Ala | tta Leu 155 | caa Gln | tta Leu | aat Asn | gct Ala | gca Ala 160 | 480 |
| gga Gly | gga Gly | atg Met | aga Arg | gtg Val 165 | gac Asp | ggt Gly | gcc Ala | aac Asn | tta Leu 170 | att Ile | ctt Leu | cat His | gta Val | gca Ala 175 | tat Tyr | 528 |
| ccc Pro | ttt Phe | gaa Glu | gca Ala 180 | atc Ile | aac Asn | cag Gln | cta Leu | aca Thr 185 | ctg Leu | cga Arg | tta Leu | gaa Glu | aac Asn 190 | glà aaa | tta Leu | 576 |
| gaa Glu | gta Val | acc Thr 195 | agc Ser | gga Gly | gga Gly | aag Lys | ctt Leu 200 | aac Asn | gtt Val | aag Lys | ttg Leu | gga Gly 205 | tca Ser | gly | ctc Leu | 624 |
| caa Gln | ttt Phe 210 | gac Asp | agt Ser | aac Asn | gga Gly | cgc Arg 215 | att Ile | gct Ala | att Ile | agt Ser | aat Asn 220 | agc Ser | aac Asn | cga Arg | act Thr | 672 |
| cga Arg 225 | agt Ser | gta Val | cca Pro | tcc Ser | ctc Leu 230 | act Thr | acc Thr | att Ile | tgg Trp | tct Ser 235 | atc Ile | tcg Ser | cct Pro | acg Thr | cct Pro 240 | 720 |
| aac Asn | tgc Cys | tcc Ser | att Ile | tat Tyr 245 | gaa Glu | acc Thr | caa Gln | gat Asp | gca Ala 250 | aac Asn | cta Leu | ttt Phe | ctt Leu | tgt Cys 255 | cta Leu | 768 |
| act Thr | aaa Lys | aac Asn | gga Gly 260 | gct Ala | cac His | gta Val | tta Leu | ggt Gly 265 | act Thr | ata Ile | aca Thr | atc Ile | aaa Lys 270 | ggt Gly | ctt Leu | 816 |
| aaa Lys | gga Gly | gca Ala 275 | ctg Leu | cgg Arg | gaa Glu | atg Met | cac His 280 | gat Asp | aac Asn | gct Ala | cta Leu | tct Ser 285 | tta Leu | aaa Lys | ctt Leu | 864 |
| ccc Pro | ttt Phe 290 | gac Asp | aat Asn | cag Gln | gga Gly | aat Asn 295 | tta Leu | ctt Leu | aac Asn | tgt Cys | gcc Ala 300 | ttg Leu | gaa Glu | tca Ser | tcc Ser | 912 |
| acc Thr 305 | tgg Trp | cgt Arg | tac Tyr | cag Gln | gaa Glu 310 | acc Thr | aac Asn | gca Ala | gtg Val | gcc Ala 315 | tct Ser | aat Asn | gcc Ala | tta Leu | aca Thr 320 | 960 |
| ttt | atg | ccc | aac | agt | aca | gtg | tat | cca | cga | aac | aaa | acc | gct | cac | ccg | 1008 |

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| Phe | Met | Pro | Asn | Ser 325 | Thr | Val | Tyr | Pro | Arg 330 | Asn | Lys | Thr | Ala | His 335 | Pro | |
|--------------|----------------------------------|------------|------------|------------|------------|-------------------|------------|------------|------------|------------|-------------------|------------|------------|------------|------------|------|
| | aac Asn | | | | | | | | | | | | | | | 1056 |
| | aac Asn | | | | | | | | | | | | | | | 1104 |
| gaa Glu | ccg Pro 370 | gga Gly | aaa Lys | cct Pro | ttt Phe | cac His 375 | cca Pro | cct Pro | acc Thr | gct Ala | gta Val 380 | ttt Phe | tgc Cys | tac Tyr | ata Ile | 1152 |
| | gaa Glu | | taa * | | | | | | | | | | | | | 1164 |
| <212 <212 | 0> 22 L> 38 2> PF 3> Ar | 37 RT | lcial | L Sec | quenc | ce | | | | | | | | | | |
| <220 <223 | 0> 8> Ad | 141 : | short | t fik | per | | | | | | | | | | | |
| |)> 22 Lys | | Thr | Arg 5 | Ile | Glu | Asp | Asp | Phe 10 | Asn | Pro | Val | Tyr | Pro 15 | Tyr | |
| | Thr | Phe | Ser 20 | Thr | Pro | Ser | Ile | Pro 25 | | Val | Ala | Pro | Pro 30 | | Val | |
| Ser | Ser | Asp 35 | Gly | Leu | Gln | Glu | Lys 40 | Pro | Pro | Gly | Val | Leu 45 | Ala | Leu | Lys | |
| Tyr | Thr 50 | Asp | Pro | Ile | Thr | Thr 55 | Asn | Ala | Lys | His | Glu 60 | Leu | Thr | Leu | Lys | |
| Leu 65 | Gly | Ser | Asn | Ile | Thr 70 | Leu | Glu | Asn | Gly | Leu 75 | Leu | Ser | Ala | Thr | Val 80 | |
| | Thr | Val | Ser | Pro 85 | Pro | Leu | Thr | Asn | Ser 90 | Asn | Asn | Ser | Leu | Gly 95 | Leu | |
| Ala | Thr | Ser | Ala 100 | | Ile | Ala | Val | Ser 105 | Ala | Asn | Ser | Leu | Thr 110 | Leu | Ala | |
| Thr | Ala | Ala 115 | | Leu | Thr | Val | Ser 120 | Asn | Asn | Gln | Leu | Ser 125 | Ile | Asn | Ala | |
| Gly | Arg 130 | | Leu | Val | Ile | Thr 135 | | Asn | Ala | Leu | Thr 140 | Val | Asn | Pro | Thr | |
| Gly 145 | Ala | Leu | Gly | Phe | Asn 150 | | Thr | Gly | Ala | Leu 155 | | Leu | Asn | Ala | Ala 160 | |
| | Gly | Met | Arg | Val 165 | | Gly | Ala | Asn | Leu 170 | Ile | Leu | His | Val | Ala 175 | Tyr | |
| Pro | Phe | Glu | Ala 180 | | Asn | Gln | Leu | Thr 185 | | Arg | Leu | Glu | Asn 190 | Gly | Leu | |
| Glu | Val | Thr 195 | | Gly | Gly | Lys | Leu 200 | | Val | Lys | Leu | Gly 205 | | Gly | Leu | |
| Gln | Phe 210 | | Ser | Asn | Gly | Arg 215 | | Ala | Ile | Ser | Asn 220 | | Asn | Arg | Thr | |
| Arg 225 | Ser | Val | Pro | Ser | Leu 230 | | Thr | Ile | Trp | Ser 235 | | Ser | Pro | Thr | Pro 240 | |
| | Cys | Ser | Ile | Tyr 245 | | Thr | Gln | Asp | Ala 250 | | Leu | Phe | Leu | Cys 255 | | |

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| Thr | Lys | Asn | Gly 260 | Ala | His | Val | Leu | Gly 265 | Thr | Ile | Thr | Ile | Lys 270 | Gly | Leu | |
|--|--|--|--|--|--|--|--|--|--|--|--|---|--|---|--|-------------------|
| Lys | Gly | Ala 275 | | Arg | Glu | Met | His 280 | | Asn | Ala | Leu | Ser 285 | | Lys | Leu | |
| Pro | Phe 290 | | Asn | Gln | Gly | Asn 295 | | Leu | Asn | Cys | Ala 300 | | Glu | Ser | Ser | |
| Thr 305 | Trp | Arg | Tyr | Gln | Glu 310 | | Asn | Ala | Val | Ala 315 | | Asn | Ala | Leu | Thr 320 | |
| | Met | Pro | Asn | Ser 325 | | Val | Tyr | Pro | Arg 330 | | Lys | Thr | Ala | His 335 | | |
| Gly | Asn | Met | Leu 340 | Ile | Gln | Ile | Ser | Pro 345 | Asn | Ile | Thr | Phe | Ser 350 | Val | Val | |
| Tyr | Asn | Glu 355 | Ile | Asn | Ser | Gly | Tyr 360 | Ala | Phe | Thr | Phe | Lys 365 | Trp | Ser | Ala | |
| Glu | Pro 370 | Gly | Lys | Pro | Phe | His 375 | Pro | Pro | Thr | Ala | Val 380 | Phe | Сув | Tyr | Ile | |
| Thr 385 | Glu | Glu | | | | | | | | | | | | | | |
| <21 | 0> 23 l> 11 2> DN | 194 | | | | | | | | | | | | | | |
| <213 | 3> A1 | rtifi | icial | l Sec | quen | ce | | | | | | | | | | |
| <220 <223 |)> 3> 41 | LsF I | RGD | | | | | | | | | | | | | |
| | L> CI 2> (1 | | . (119 | 94) | | | | | | | | | | | | |
| |)> 23 aaa | | 200 | 202 | 2++ | ~ 33 | C 2 C | asa. | tta | 224 | aaa | at a | toa | aaa | ++ | 48 |
| | | uqu | acc | aga | acc | | | | | | | | | | | # 0 |
| | Lys | | | Arg 5 | Ile | GIu | Asp | Asp | 10 | Asn | Pro | val | тут | 15 | Tyr | |
| Met 1 gac | | Arg | Thr | 5 act | ccc | agc | atc | aaa | 10 tat | gta | gct | ccg | ccc | 15 ttc | gtt | 96 |
| Met 1 gac Asp | Lys | Arg ttc Phe gac | tca Ser 20 | 5 act Thr | ccc Pro | agc Ser gaa | atc Ile | ccc Pro 25 | 10 tat Tyr cca | gta Val gga | gct Ala gtt | ccg Pro tta | ccc Pro 30 | 15 ttc Phe ctc | gtt Val | 96 144 |
| Met 1 gac Asp tct Ser | Lys acc Thr | Arg ttc Phe gac Asp 35 gac | Thr tca Ser 20 ggg Gly | act Thr tta Leu | ccc Pro cag Gln | agc Ser gaa Glu | atc Ile aaa Lys 40 | ccc Pro 25 ccc Pro | tat Tyr cca Pro | gta Val gga Gly cat | gct Ala gtt Val | ccg Pro tta Leu 45 | ccc Pro 30 gca Ala | 15 ttc Phe ctc Leu | gtt Val aag Lys | |
| Met 1 gac Asp tct Ser tac Tyr | acc Thr tct Ser act Thr | ttc Phe gac Asp 35 gac Asp | Thr tca ser 20 ggg Gly ccc Pro | act Thr tta Leu att Ile | ccc Pro cag Gln act Thr | agc Ser gaa Glu acc Thr 55 | atc Ile aaa Lys 40 aat Asn | ccc Pro 25 ccc Pro gct Ala | tat Tyr cca Pro aag Lys | gta Val gga Gly cat His | gct Ala gtt Val gag Glu 60 | ccg Pro tta Leu 45 ctt Leu | ccc Pro 30 gca Ala act Thr | ttc Phe ctc Leu tta Leu | gtt Val aag Lys aaa Lys | 144 |
| Met 1 gac Asp tct Ser tac Tyr ctt Leu 65 ccc | acc Thr tct Ser act Thr 50 | ttc Phe gac Asp 35 gac Asp agc ser | Thr tca Ser 20 ggg Gly ccc Pro aac Asn | 5 act Thr tta Leu att Ile ata Ile cct | ccc Pro cag Gln act Thr act Thr | agc Ser gaa Glu acc Thr 55 tta Leu | atc Ile aaaa Lys 40 aat Asn gaa Glu aca | ccc Pro 25 ccc Pro gct Ala aat Asn | tat Tyr cca Pro aag Lys ggg Gly | gta Val gga Gly cat His tta Leu 75 | gct Ala gtt Val gag Glu 60 ctt Leu | ccg Pro tta Leu 45 ctt Leu tcg ser | ccc Pro 30 gca Ala act Thr | ttc Phe ctc Leu tta Leu aca Thr | gtt Val aag Lys aaa Lys gtt Val 80 | 144 192 |
| Met 1 gac Asp tct Ser tac Tyr ctt Leu 65 ccc Pro | Lys acc Thr tct Ser act Thr 50 gga Gly act | Arg ttc Phe gac Asp 35 gac Asp agc ser gtt Val tcc | Thr tca Ser 20 999 Gly ccc Pro aac Asn tct Ser | act Thr tta Leu att Ile ata Ile cct Pro 85 | ccc Pro cag Gln act Thr 70 ccc Pro | agc Ser gaa Glu acc Thr 55 tta Leu ctt Leu | atc Ile aaa Lys 40 aat Asn gaa Glu aca Thr | ccc Pro 25 ccc Pro gct Ala aat Asn aac Asn | tat Tyr cca Pro aag Lys ggg Gly agt ser 90 | gta Val gga Gly cat His tta Leu 75 aac Asn | gct Ala gtt Val gag Glu 60 ctt Leu aac Asn | ccg Pro tta Leu 45 ctt Leu tcg ser tcc | ccc Pro 30 gca Ala act Thr gcc Ala ctg Leu | ttc Phe ctc Leu tta Leu aca Thr ggt Gly 95 ttg | gtt Val aag Lys aaa Lys gtt Val 80 tta Leu | 144 192 240 |

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| Thr | Ala | Ala 115 | Pro | Leu | Thr | Val | Ser 120 | Asn | Asn | Gln | Leu | Ser 125 | Ile | Asn | Ala | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------------|-------------------|------|
| ggc | aga Arg 130 | Gly | tta Leu | gtt Val | ata Ile | act Thr 135 | aac Asn | aat Asn | gcc Ala | tta Leu | aca Thr 140 | gtt Val | aat Asn | cct Pro | acc Thr | 432 |
| gga Gly 145 | gcg Ala | cta Leu | ggt Gly | ttc Phe | aat Asn 150 | aac Asn | aca Thr | gga Gly | gct Ala | tta Leu 155 | caa Gln | tta Leu | aat Asn | gct Ala | gca Ala 160 | 480 |
| gga Gly | gga Gly | atg Met | aga Arg | gtg Val 165 | gac Asp | ggt Gly | gcc Ala | aac Asn | tta Leu 170 | att Ile | ctt Leu | cat His | gta Val | gca Ala 175 | tat Tyr | 528 |
| ccc Pro | ttt Phe | gaa Glu | gca Ala 180 | atc Ile | aac Asn | cag Gln | cta Leu | aca Thr 185 | ctg Leu | cga Arg | tta Leu | gaa Glu | aac Asn 190 | gl ^à aaa | tta Leu | 576 |
| gaa Glu | gta Val | acc Thr 195 | agc Ser | gga Gly | gga Gly | aag Lys | ctt Leu 200 | aac Asn | gtt Val | aag Lys | ttg Leu | gga Gly 205 | tca Ser | ggc Gly | ctc Leu | 624 |
| caa Gln | ttt Phe 210 | gac Asp | agt Ser | aac Asn | gga Gly | cgc Arg 215 | att Ile | gct Ala | att Ile | agt Ser | aat Asn 220 | agc Ser | aac Asn | cga Arg | act Thr | 672 |
| cga Arg 225 | agt Ser | gta Val | cca Pro | tcc Ser | ctc Leu 230 | act Thr | acc Thr | att Ile | tgg Trp | tct Ser 235 | atc Ile | tcg Ser | cct Pro | acg Thr | cct Pro 240 | 720 |
| aac Asn | tgc Cys | tcc Ser | att Ile | tat Tyr 245 | gaa Glu | acc Thr | caa Gln | gat Asp | gca Ala 250 | aac Asn | cta Leu | ttt Phe | ctt Leu | tgt Cys 255 | cta Leu | 768 |
| act Thr | aaa Lys | aac Asn | gga Gly 260 | gct Ala | cac His | gta Val | tta Leu | ggt Gly 265 | act Thr | ata Ile | aca Thr | atc Ile | aaa Lys 270 | ggt Gly | ctt Leu | 816 |
| aaa Lys | gga Gly | gca Ala 275 | ctg Leu | cgg Arg | gaa Glu | atg Met | cac His 280 | gat Asp | aac Asn | gct Ala | cta Leu | tct Ser 285 | tta Leu | aaa Lys | ctt Leu | 864 |
| ccc Pro | ttt Phe 290 | gac Asp | aat Asn | cag Gln | gga Gly | aat Asn 295 | tta Leu | ctt Leu | aac Asn | tgt Cys | gcc Ala 300 | ttg Leu | gaa Glu | tca Ser | tcc Ser | 912 |
| acc Thr 305 | tgg Trp | cgt Arg | tac Tyr | cag Gln | gaa Glu 310 | acc Thr | aac Asn | gca Ala | gtg Val | gcc Ala 315 | tct Ser | aat Asn | gcc Ala | tta Leu | aca Thr 320 | 960 |
| ttt Phe | atg Met | ccc Pro | aac Asn | agt Ser 325 | aca Thr | gtg Val | tat Tyr | cca Pro | cga Arg 330 | aac Asn | aaa Lys | acc Thr | gct Ala | cac His 335 | ccg Pro | 1008 |
| ggc Gly | aac Asn | atg Met | ctc Leu 340 | atc Ile | caa Gln | atc Ile | tcg Ser | cct Pro 345 | aac Asn | atc Ile | acc Thr | ttc Phe | agt Ser 350 | gtc Val | gtc Val | 1056 |
| tac Tyr | aac Asn | gag Glu | ata Ile | aac Asn | tgt Cys | gat Asp | tgt Cys | cgt Arg | ggt Gly | gat Asp | tgt Cys | ttt Phe | tgt Cys | act Thr | agt Ser | 1104 |

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355 360 365 ggg tat gct ttt act ttt aaa tgg tca gcc gaa ccg gga aaa cct ttt 1152 Gly Tyr Ala Phe Thr Phe Lys Trp Ser Ala Glu Pro Gly Lys Pro Phe cac cca cct acc gct gta ttt tgc tac ata act gaa gaa taa 1194 His Pro Pro Thr Ala Val Phe Cys Tyr Ile Thr Glu Glu * 390 <210> 24 <211> 397 <212> PRT <213> Artificial Sequence <220> <223> 41sFRGD <400> 24 Met Lys Arg Thr Arg Ile Glu Asp Asp Phe Asn Pro Val Tyr Pro Tyr 1.0 Asp Thr Phe Ser Thr Pro Ser Ile Pro Tyr Val Ala Pro Pro Phe Val 20 25 30 Ser Ser Asp Gly Leu Gln Glu Lys Pro Pro Gly Val Leu Ala Leu Lys 4.0 45 Tyr Thr Asp Pro Ile Thr Thr Asn Ala Lys His Glu Leu Thr Leu Lys 50 55 60 Leu Gly Ser Asn Ile Thr Leu Glu Asn Gly Leu Leu Ser Ala Thr Val 70 75 Pro Thr Val Ser Pro Pro Leu Thr Asn Ser Asn Asn Ser Leu Gly Leu 85 90 Ala Thr Ser Ala Pro Ile Ala Val Ser Ala Asn Ser Leu Thr Leu Ala 100 105 Thr Ala Ala Pro Leu Thr Val Ser Asn Asn Gln Leu Ser Ile Asn Ala 115 120 125 Gly Arg Gly Leu Val Ile Thr Asn Asn Ala Leu Thr Val Asn Pro Thr 130 135 140 Gly Ala Leu Gly Phe Asn Asn Thr Gly Ala Leu Gln Leu Asn Ala Ala 1.50 155 160 Gly Gly Met Arg Val Asp Gly Ala Asn Leu Ile Leu His Val Ala Tyr 165 170 Pro Phe Glu Ala Ile Asn Gln Leu Thr Leu Arg Leu Glu Asn Gly Leu 180 185 190 Glu Val Thr Ser Gly Gly Lys Leu Asn Val Lys Leu Gly Ser Gly Leu 195 200 Gln Phe Asp Ser Asn Gly Arg Ile Ala Ile Ser Asn Ser Asn Arg Thr 210 215 220 Arg Ser Val Pro Ser Leu Thr Thr Ile Trp Ser Ile Ser Pro Thr Pro 230 235 Asn Cys Ser Ile Tyr Glu Thr Gln Asp Ala Asn Leu Phe Leu Cys Leu 245 250 255 Thr Lys Asn Gly Ala His Val Leu Gly Thr Ile Thr Ile Lys Gly Leu 260 265 270 Lys Gly Ala Leu Arg Glu Met His Asp Asn Ala Leu Ser Leu Lys Leu 280 275 285 Pro Phe Asp Asn Gln Gly Asn Leu Leu Asn Cys Ala Leu Glu Ser Ser 295 300 Thr Trp Arg Tyr Gln Glu Thr Asn Ala Val Ala Ser Asn Ala Leu Thr 310 315

Phe Met Pro Asn Ser Thr Val Tyr Pro Arg Asn Lys Thr Ala His Pro

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| FIIC | Mec | FIO | Maii | 325 | 7117 | vaı | тут | FIO | 330 | Apm | цур | 7177 | та | 335 | FIO | |
|--------------|----------------------------------|-------------------|-------------------|-----------------|------------|-------------------|-------------------|-------------------|------------------|------------|-------------------|-------------------|-------------------|------------------|------------|-----|
| Gly | Asn | Met | Leu 340 | | Gln | Ile | Ser | Pro 345 | | Ile | Thr | Phe | Ser 350 | Val | Val | |
| Tyr | Asn | Glu 355 | | Asn | Cys | Asp | Cys 360 | | Gly | Asp | Cys | Phe 365 | | Thr | Ser | |
| Gly | Tyr 370 | | Phe | Thr | Phe | Lys 375 | | Ser | Ala | Glu | Pro 380 | | Lys | Pro | Phe | |
| His 385 | Pro | Pro | Thr | Ala | Val 390 | Phe | Cys | Tyr | Ile | Thr 395 | | Glu | | | | |
| <211 <212 | 0> 25 L> 17 2> DN 3> An | 737 VA | icial | l Sed | quenc | ce | | | | | | | | | | |
| <220 <223 |)> 3> Ac | d5 p€ | entor | n | | | | | | | | | | | | |
| | L> CI 2> (1 | | . (173 | 37) | | | | | | | | | | | | |
| atg |)> 25 cgg Arg | cgc | gcg Ala | gcg Ala 5 | atg Met | tat Tyr | gag Glu | gaa Glu | ggt Gly 10 | cct Pro | cct Pro | ccc Pro | tcc Ser | tac Tyr 15 | gag Glu | 48 |
| agt Ser | gtg Val | gtg Val | agc Ser 20 | gcg Ala | gcg Ala | cca Pro | gtg Val | gcg Ala 25 | gcg Ala | gcg Ala | ctg Leu | ggt Gly | tct Ser 30 | ccc Pro | ttc Phe | 96 |
| gat Asp | gct Ala | ccc Pro 35 | ctg Leu | gac Asp | ccg Pro | ccg Pro | ttt Phe 40 | gtg Val | cct Pro | ccg Pro | cgg Arg | tac Tyr 45 | ctg Leu | cgg Arg | cct Pro | 144 |
| | | | | | | | | | | | | | | cta Leu | | 192 |
| | | | | | | | | | | | | | | gtg Val | | 240 |
| | | | | | | | | | | | | | | gtc Val 95 | | 288 |
| caa Gln | aac Asn | aat Asn | gac Asp 100 | tac Tyr | agc Ser | ccg Pro | gly aaa | gag Glu 105 | gca Ala | agc Ser | aca Thr | cag Gln | acc Thr 110 | atc Ile | aat Asn | 336 |
| ctt Leu | gac Asp | gac Asp 115 | cgg Arg | tcg Ser | cac His | tgg Trp | ggc Gly 120 | gly ggc | gac Asp | ctg Leu | aaa Lys | acc Thr 125 | atc Ile | ctg Leu | cat His | 384 |
| acc Thr | aac Asn 130 | atg Met | cca Pro | aat Asn | gtg Val | aac Asn 135 | gag Glu | ttc Phe | atg Met | ttt Phe | acc Thr 140 | aat Asn | aag Lys | ttt Phe | aag Lys | 432 |
| gcg | cgg | gtg | atg | gtg | tcg | cgc | ttg | cct | act | aag | gac | aat | cag | gtg | gag | 480 |

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| Ala 145 | Arg | Val | Met | Val | Ser 150 | Arg | Leu | Pro | Thr | Lys 155 | Asp | Asn | Gln | Val | Glu 160 | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------------|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| | | | | | | gag Glu | | | | | | | | | | 528 |
| | | | | | | ctt Leu | | | | | | | | | | 576 |
| ttg Leu | aaa Lys | gtg Val 195 | ggc Gly | aga Arg | cag Gln | aac Asn | 500 Gl ^A 333 | gtt Val | ctg Leu | gaa Glu | agc Ser | gac Asp 205 | atc Ile | gly aaa | gta Val | 624 |
| | | | | | | ttc Phe 215 | | | | | | | | | | 672 |
| | | | | | | tat Tyr | | | | | | | | | | 720 |
| att Ile | ttg Leu | ctg Leu | cca Pro | gga Gly 245 | tgc Cys | gl ^à aaa | gtg Val | gac Asp | ttc Phe 250 | acc Thr | cac His | agc Ser | cgc Arg | ctg Leu 255 | agc Ser | 768 |
| aac Asn | ttg Leu | ttg Leu | ggc Gly 260 | atc Ile | cgc Arg | aag Lys | cgg Arg | caa Gln 265 | ccc Pro | ttc Phe | cag Gln | gag Glu | ggc Gly 270 | ttt Phe | agg Arg | 816 |
| atc Ile | acc Thr | tac Tyr 275 | gat Asp | gat Asp | ctg Leu | gag Glu | ggt Gly 280 | ggt Gly | aac Asn | att Ile | ccc Pro | gca Ala 285 | ctg Leu | ttg Leu | gat Asp | 864 |
| | | | | | | agc Ser 295 | | | | | | | | | | 912 |
| ggt Gly 305 | ggc Gly | gca Ala | ggc Gly | ggc Gly | agc Ser 310 | aac Asn | agc Ser | agt Ser | ggc Gly | agc Ser 315 | Gly ggc | gcg Ala | gaa Glu | gag Glu | aac Asn 320 | 960 |
| tcc Ser | aac Asn | gcg Ala | gca Ala | gcc Ala 325 | gcg Ala | gca Ala | atg Met | cag Gln | ccg Pro 330 | gtg Val | gag Glu | gac Asp | atg Met | aac Asn 335 | gat Asp | 1008 |
| agc Ser | cgc Arg | ggc Gly | tac Tyr 340 | ccc Pro | tac Tyr | gac Asp | gtg Val | ccc Pro 345 | gac Asp | tac Tyr | gcg Ala | ggc Gly | acc Thr 350 | agc Ser | gcc Ala | 1056 |
| aca Thr | cgg Arg | gct Ala 355 | gag Glu | gag Glu | aag Lys | cgc Arg | gct Ala 360 | gag Glu | gcc Ala | gaa Glu | gca Ala | gcg Ala 365 | gcc Ala | gaa Glu | gct Ala | 1104 |
| gcc Ala | gcc Ala 370 | ccc Pro | gct Ala | gcg Ala | caa Gln | ccc Pro 375 | gag Glu | gtc Val | gag Glu | aag Lys | cct Pro 380 | cag Gln | aag Lys | aaa Lys | ccg Pro | 1152 |
| gtg Val | atc Ile | aaa Lys | ccc Pro | ctg Leu | aca Thr | gag Glu | gac Asp | agc Ser | aag Lys | aaa Lys | cgc Arg | agt Ser | tac Tyr | aac Asn | cta Leu | 1200 |

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| 385 | 390 | 395 | 400 |
|---|---|---------------------|--------------------------------|
| ata agc aat gac agc Ile Ser Asn Asp Ser 405 | acc ttc acc cag tag Thr Phe Thr Gln Ty: | r Arg Ser Trp Tyr I | ctt gca 1248 Leu Ala 415 |
| tac aac tac ggc gac Tyr Asn Tyr Gly Asp 420 | | | |
| tgc act cct gac gta Cys Thr Pro Asp Val 435 | | | |
| | gac ccc gtg acc tte Asp Pro Val Thr Pho 455 | | |
| | gtg ggc gcc gag ctg Val Gly Ala Glu Le 470 | | |
| | cag gcc gtc tac tc Gln Ala Val Tyr Se 49 | r Gln Leu Ile Arg (| |
| | gtg ttc aat cgc tt Val Phe Asn Arg Pho 505 | | |
| | ccc acc atc acc ac Pro Thr Ile Thr Th 520 | | |
| | ggg acg cta ccg ct Gly Thr Leu Pro Le 535 | | |
| | att act gac gcc ag Ile Thr Asp Ala Ar 550 | | |
| | g ggc ata gtc tcg cc Gly Ile Val Ser Pr 57 | o Arg Val Leu Ser S | |
| act ttt tga Thr Phe * | | | 1737 |
| <210> 26 <211> 577 <212> PRT <213> Artificial Se | equence | | |
| <220> <223> Ad5 penton | | | |

 ${<}400{>}\ 26$ Arg Arg Ala Met Tyr Glu Glu Gly Pro Pro Pro Ser Tyr Glu Ser

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| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
|-----------|-----------|-----------|-----------|-----|-----------|-----------|--------------|-----------|-----|------------|-----------|-----------|-----------|-----|-----------|
| | Val | Ser | Ala 20 | | Pro | Val | Ala | Ala 25 | | Leu | Gly | Ser | Pro 30 | | Asp |
| Ala | Pro | Leu 35 | qaA | Pro | Pro | Phe | Val 40 | Pro | Pro | Arg | Tyr | Leu 45 | Arg | Pro | Thr |
| Gly | Gly 50 | Arg | Asn | Ser | Ile | Arg 55 | Tyr | Ser | Glu | Leu | Ala 60 | Pro | Leu | Phe | Asp |
| Thr 65 | Thr | Arg | Val | Tyr | Leu 70 | Val | qaA | Asn | Lys | Ser 75 | Thr | qaA | Val | Ala | Ser 80 |
| | | | | 85 | | | Ser | | 90 | | | | | 95 | |
| | | | 100 | | | | Glu | 105 | | | | | 110 | | |
| | | 115 | | | | | Gly 120 | | | | | 125 | | | |
| | 130 | | | | | 135 | Phe | | | | 140 | | | | |
| 145 | | | | | 150 | | Pro | | | 155 | | | | | 160 |
| _ | | | | 165 | | | Thr | | 170 | | | | | 175 | |
| | | | 180 | | | | Asn | 185 | | | | | 190 | | |
| | | 195 | | | | | Val 200 | | | | | 205 | | | |
| | 210 | | | | | 215 | Leu - | | | | 220 | | | | |
| 225 | | | | | 230 | | Asn - | | | 235 | | | | | 240 |
| | | | | 245 | | | Asp | | 250 | | | | | 255 | |
| | | | 260 | | | | Gln | 265 | | | | | 270 | | |
| | | 275 | | | | | Gly 280 | | | | | 285 | | | |
| | 290 | | | | | 295 | Lys | | | | 300 | | | | |
| 305 | | | | | 310 | | Ser | | | 315 | | | | | 320 |
| | | | | 325 | | | Gln | | 330 | | | | | 335 | |
| | | | 340 | | | | Pro | 345 | | | | | 350 | | |
| _ | | 355 | | - | | | Glu 360 | | | | | 365 | | | |
| | 370 | | | | | 375 | Val | | | | 380 | | | | |
| 385 | - | | | | 390 | | Ser | | | 395 | | | | _ | 400 |
| | | - | | 405 | | | Gln | _ | 410 | | | | | 415 | |
| | | | 420 | | | | Gly Ser | 425 | | | | | 430 | | |
| | | 435 | Gln | | | | 440 | | | | | 445 | | Ile | |
| _ | 450 | | | | | 455 | Glu | | | | 460 | | | | |
| 465 | | | | | 470 | | Tyr | | | 475 | | | | | 480 |
| FIIG | тХт | won | цар | 485 | | Val | ~ <u>}</u> _ | UCI | 490 | بال ت المد | 440 | 9 | <u></u> | 495 | |

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| Ser Le | eu Thr | His 500 | Val | Phe | Asn | Arg | Phe 505 | Pro | Glu | Asn | Gln | Ile 510 | Leu | Ala | |
|------------------|------------------|------------|------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|
| Arg Pi | ro Pro 515 | | Pro | Thr | Ile | Thr 520 | Thr | Val | Ser | Glu | Asn 525 | ۷al | Pro | Ala | |
| Leu Th | nr Asp 30 | His | Gly | Thr | Leu 535 | Pro | Leu | Arg | Asn | Ser 540 | Ile | Gly | Gly | Val | |
| Gln Ai 545 | rg Val | Thr | Ile | Thr 550 | Asp | Ala | Arg | Arg | Arg 555 | Thr | Cys | Pro | Tyr | Val 560 | |
| Tyr Ly | ys Ala | . Leu | Gly 565 | Ile | Val | Ser | Pro | Arg 570 | | Leu | Ser | Ser | Arg 575 | | |
| Phe | | | | | | | | | | | | | | | |
| 010 | 0.5 | | | | | | | | | | | | | | |
| <210><211><212> | 1773 | | | | | | | | | | | | | | |
| <213> | | icia | l Sed | quen | ce | | | | | | | | | | |
| <220> <223> | 5TS35 | Н | | | | | | | | | | | | | |
| <221> <222> | | . (17 | 73) | | | | | | | | | | | | |
| <400> atg aa | | qca | aqa | cca | tct | qaa | gat | acc | ttc | aac | ccc | ata | tat | cca | 48 |
| Met Ly 1 | ys Arg | Āla | Arg 5 | Pro | Ser | Ğlu | Āsp | Thr 10 | Phe | Asn | Pro | Val | Tyr 15 | Pro | |
| tat ga Tyr As | | | | | | | | | | | | | | | 96 |
| 2 | L | 20 | | 2 | | | 25 | | | | | 30 | | | |
| ttt gt Phe Va | al Ser | Pro | aat Asn | gly ggg | ttt Phe | caa Gln | gag Glu | agt Ser | ccc Pro | cct Pro | gly aaa | gta Val | ctc Leu | tct Ser | 144 |
| | 35 | | | | | 40 | | | | | 45 | | | | |
| ttg co | g Leu | | | | Leu | | | | | Gly | | | | | 192 |
| aaa at | 50 50 | 220 | aaa | ata | 55 | ata | asa | asa | aaa | 60 | 220 | att | 200 | taa | 240 |
| Lys Me | | | | | | | | | | | | | | | 240 |
| caa aa | at qta | acc | act | | agc | cca | cct | ctc | | aaa | acc | aaq | tca | | 288 |
| Gln As | | | | | | | | | | | | | | | |
| ata aa | | | | | | | | | | | | | | | 336 |
| Ile As | sn Leu | 100 | Ile | Ser | Ala | Pro | Leu 105 | Thr | Val | Thr | Ser | Glu 110 | Ala | Leu | |
| act gt Thr Va | g gct | gcc | gcc | gca 11 a | cct | cta | atg Met | gtc | gcg | ggc | aac | aca | ctc | acc | 384 |
| T11T VC | 115 | | лта | AT CL | 110 | 120 | MEC | val | ATA | GTÀ | 125 | 7 117 | มอน | T-11T | |
| atg ca Met Gl | aa tca ln Ser | cag Gln | gcc Ala | ccg Pro | cta Leu | acc Thr | gtg Val | cac His | gac Asp | tcc Ser | aaa Lys | ctt Leu | agc Ser | att Ile | 432 |
| 13 | | | | | 135 | | | | - | 140 | - | | | | |

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| | acc Thr | | | | | | | | | | | | | | | 480 |
|------------|-------------------|-------------------|-------------------|-------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|------|
| | tca Ser | | | | | | | | | | | | | | | 528 |
| | tca Ser | | | | | | | | | | | | | | | 576 |
| aaa Lys | gag Glu | ccc Pro 195 | att Ile | tat Tyr | aca Thr | caa Gln | aat Asn 200 | gga Gly | aaa Lys | cta Leu | gga Gly | cta Leu 205 | aag Lys | tac Tyr | gly aaa | 624 |
| | cct Pro 210 | | | | | | | | | | | | | | | 672 |
| | cca Pro | | | | | | | | | | | | | | | 720 |
| gga Gly | gcc Ala | ttg Leu | ggt Gly | ttt Phe 245 | gat Asp | tca Ser | caa Gln | gly ggc | aat Asn 250 | atg Met | caa Gln | ctt Leu | aat Asn | gta Val 255 | gca Ala | 768 |
| gga Gly | gga Gly | cta Leu | agg Arg 260 | att Ile | gat Asp | tct Ser | caa Gln | aac Asn 265 | aga Arg | cgc Arg | ctt Leu | ata Ile | ctt Leu 270 | gat Asp | gtt Val | 816 |
| agt Ser | tat Tyr | ccg Pro 275 | ttt Phe | gat Asp | gct Ala | caa Gln | aac Asn 280 | caa Gln | cta Leu | aat Asn | cta Leu | aga Arg 285 | cta Leu | gga Gly | cag Gln | 864 |
| ggc Gly | cct Pro 290 | ctt Leu | ttt Phe | ata Ile | aac Asn | tca Ser 295 | gcc Ala | cac His | aac Asn | ttg Leu | gat Asp 300 | att Ile | aac Asn | tac Tyr | aac Asn | 912 |
| | Gly ggc | | | | | | | | | | | | | | | 960 |
| gtt Val | aac Asn | cta Leu | agc Ser | act Thr 325 | gcc Ala | aag Lys | gly aaa | ttg Leu | atg Met 330 | ttt Phe | gac Asp | gct Ala | aca Thr | gcc Ala 335 | ata Ile | 1008 |
| gcc Ala | att Ile | aat Asn | gca Ala 340 | gga Gly | gat Asp | gly aaa | ctt Leu | gaa Glu 345 | ttt Phe | ggt Gly | tca Ser | cct Pro | aat Asn 350 | gca Ala | cca Pro | 1056 |
| aac Asn | aca Thr | aat Asn 355 | ccc Pro | ctc Leu | aaa Lys | aca Thr | aaa Lys 360 | att Ile | ggc Gly | cat His | ggc Gly | cta Leu 365 | gaa Glu | ttt Phe | gat Asp | 1104 |
| tca Ser | aac Asn 370 | aag Lys | gct Ala | atg Met | gtt Val | cct Pro 375 | aaa Lys | cta Leu | gga Gly | act Thr | ggc Gly 380 | ctt Leu | agt Ser | ttt Phe | gac Asp | 1152 |

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| agc Ser 385 | aca Thr | ggt Gly | gcc Ala | att Ile | aca Thr 390 | gta Val | gga Gly | aac Asn | aaa Lys | aat Asn 395 | aat Asn | gat Asp | aag Lys | cta Leu | act Thr 400 | 1200 |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| ttg Leu | tgg Trp | acc Thr | gga Gly | ata Ile 405 | aac Asn | cct Pro | cca Pro | cct Pro | aac Asn 410 | tgt Cys | caa Gln | att Ile | gtg Val | gaa Glu 415 | aac Asn | 1248 |
| act Thr | aat Asn | aca Thr | aat Asn 420 | gat Asp | ggc Gly | aaa Lys | ctt Leu | act Thr 425 | tta Leu | gta Val | tta Leu | gta Val | aaa Lys 430 | aat Asn | gga Gly | 1296 |
| gly aaa | ctt Leu | gtt Val 435 | aat Asn | ggc Gly | tac Tyr | gtg Val | tct Ser 440 | cta Leu | gtt Val | ggt Gly | gta Val | tca Ser 445 | gac Asp | act Thr | gtg Val | 1344 |
| aac Asn | caa Gln 450 | atg Met | ttc Phe | aca Thr | caa Gln | aag Lys 455 | aca Thr | gca Ala | aac Asn | atc Ile | caa Gln 460 | tta Leu | aga Arg | tta Leu | tat Tyr | 1392 |
| | | | | | aat Asn 470 | | | | | | | | | | | 1440 |
| cca Pro | ctt Leu | aaa Lys | aat Asn | aaa Lys 485 | tct Ser | tct Ser | aca Thr | gcg Ala | acc Thr 490 | agt Ser | gaa Glu | act Thr | gta Val | gcc Ala 495 | agc Ser | 1488 |
| agc Ser | aaa Lys | gcc Ala | ttt Phe 500 | atg Met | cca Pro | agt Ser | act Thr | aca Thr 505 | gct Ala | tat Tyr | ccc Pro | ttc Phe | aac Asn 510 | acc Thr | act Thr | 1536 |
| act Thr | agg Arg | gat Asp 515 | agt Ser | gaa Glu | aac Asn | tac Tyr | att Ile 520 | cat His | gga Gly | ata Ile | tgt Cys | tac Tyr 525 | tac Tyr | atg Met | act Thr | 1584 |
| agt Ser | tat Tyr 530 | gat Asp | aga Arg | agt Ser | cta Leu | ttt Phe 535 | ccc Pro | ttg Leu | aac Asn | att Ile | tct Ser 540 | ata Ile | atg Met | cta Leu | aac Asn | 1632 |
| agc Ser 545 | cgt Arg | atg Met | att Ile | tct Ser | tcc Ser 550 | aat Asn | gtt Val | gcc Ala | tat Tyr | gcc Ala 555 | ata Ile | caa Gln | ttt Phe | gaa Glu | tgg Trp 560 | 1680 |
| aat Asn | cta Leu | aat Asn | gca Ala | agt Ser 565 | gaa Glu | tct Ser | cca Pro | gaa Glu | agc Ser 570 | aac Asn | ata Ile | gct Ala | acg Thr | ctg Leu 575 | acc Thr | 1728 |
| aca Thr | tcc Ser | ccc Pro | ttt Phe 580 | ttc Phe | ttt Phe | tct Ser | tac Tyr | att Ile 585 | aca Thr | gaa Glu | gac Asp | gac Asp | gaa Glu 590 | taa * | | 1773 |

<210> 28 <211> 590 <212> PRT <213> Artificial Sequence

<220> <223> 5TS35H

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Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser Glu Thr Val Ala Ser

490 485 Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr Pro Phe Asn Thr Thr 505 500 Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile Cys Tyr Tyr Met Thr 525 520 515 Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile Ser Ile Met Leu Asn 535 540 Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala Ile Gln Phe Glu Trp 555 550 Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn Ile Ala Thr Leu Thr 570 565 Thr Ser Pro Phe Phe Ser Tyr Ile Thr Glu Asp Asp Glu 585 <210> 29 <211> 945 <212> DNA <213> Artificial Sequence <220> <223> 35TS5H <221> CDS <222> (1) ... (945) <400> 29 atg acc aag aga gtc cgg ctc agt gac tcc ttc aac cct gtc tac ccc 48 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt 96 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu 144 40 35 192 aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys 55 240 gtg gga ggg gga ctt aca gtg gat gac act gat ggt acc tta caa gaa Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu aac ata cgt gct aca gca ccc att act aaa aat aat cac tct gta gaa 288 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu cta tcc att gga aat gga tta gaa act caa aac aat aaa cta tgt gcc Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala 100 aaa ttg gga aat ggg tta aaa ttt aac aac ggt gac att tgt ata aag Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys gat agt att aac acc tta tgg act aca cca gct cca tct cct aac tgt 432

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| Asp | Ser 130 | Ile | Asn | Thr | Leu | Trp 135 | Thr | Thr | Pro | Ala | Pro 140 | Ser | Pro | Asn | Cys | |
|-------------------|----------------------------------|-------------------|-------------------|------------|-------------------|------------|-------------------|-------------------|------------|-------------------|------------|-------------------|-------------------|------------|-------------------|-----|
| | | | gca Ala | | | | | | | | | | | | | 480 |
| | | | caa Gln | | | | | | | | | | | | | 528 |
| | | | cca Pro 180 | | | | | | | | | | | | | 576 |
| aga Arg | ttt Phe | gac Asp 195 | gaa Glu | aat Asn | gga Gly | gtg Val | cta Leu 200 | cta Leu | aac Asn | aat Asn | tcc Ser | ttc Phe 205 | ctg Leu | gac Asp | cca Pro | 624 |
| | | | aac Asn | | | | | | | | | | | | | 672 |
| aca Thr 225 | aac Asn | gct Ala | gtt Val | gga Gly | ttt Phe 230 | atg Met | cct Pro | aac Asn | cta Leu | tca Ser 235 | gct Ala | tat Tyr | cca Pro | aaa Lys | tct Ser 240 | 720 |
| | | | act Thr | | | | | | | | | | | | | 768 |
| gga Gly | gac Asp | aaa Lys | act Thr 260 | aaa Lys | cct Pro | gta Val | aca Thr | cta Leu 265 | acc Thr | att Ile | aca Thr | cta Leu | aac Asn 270 | ggt Gly | aca Thr | 816 |
| | | | gga Gly | | | | | | | | | | | | | 864 |
| | | | tct Ser | | | | | | | | | | | | | 912 |
| | | | ttt Phe | | | | | | | taa * | | | | | | 945 |
| <211 <212 |)> 30 .> 31 ?> PR }> Ar | .4 RT | icial | . Sec | quenc | ce | | | | | | | | | | |
| <220 <223 |)> 3> 35 | TS51 | I | | | | | | | | | | | | | |
| |)> 30 Thr | | Arg | Val | Arg | Leu | Ser | Asp | Ser 10 | Phe | Asn | Pro | Val | Tyr 15 | Pro | |
| | Glu | Asp | Glu 20 | Ser | Thr | Ser | Gln | His 25 | | Phe | Ile | Asn | Pro 30 | | Phe | |

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```
Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
        35
                            40
Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys
                        55
Val Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
                    70
                                        75
Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
                                  90
Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala
            100
                                105
                                                    110
Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
        115
                            120
                                                125
Asp Ser Ile Asn Thr Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys
                        135
                                            140
Arg Leu Asn Ala Glu Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys
                    150
                                        155
Cys Gly Ser Gln Ile Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly
                165
                                    170
                                                        175
Ser Leu Ala Pro Ile Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile
                                185
                                                    190
Arg Phe Asp Glu Asn Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro
                         200
                                                205
Glu Tyr Trp Asn Phe Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr
                        215
                                            220
Thr Asn Ala Val Gly Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser
                    230
                                        235
His Gly Lys Thr Ala Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn
                245
                                    250
Gly Asp Lys Thr Lys Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr
            260
                                265
                                                    270
Gln Glu Thr Gly Asp Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser
                            280
                                               285
Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser
                        295
Ser Tyr Thr Phe Ser Tyr Ile Ala Gln Glu
                    310
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<212> DNA
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atg tca aag agg ctc cgg gtg gaa gat gac ttc aac ccc gtc tac ccc
                                                                  48
Met Ser Lys Arg Leu Arg Val Glu Asp Asp Phe Asn Pro Val Tyr Pro
tat ggc tac gcg cgg aat cag aat atc ccc ttc ctc act ccc ccc ttt
                                                                  96
Tyr Gly Tyr Ala Arg Asn Gln Asn Ile Pro Phe Leu Thr Pro Pro Phe
gtc tcc tcc gat gga ttc aaa aac ttc ccc cct ggg gta ctg tca ctc
                                                                  144
Val Ser Ser Asp Gly Phe Lys Asn Phe Pro Pro Gly Val Leu Ser Leu
aaa ctg gct gat cca atc acc att acc aat ggg gat gta tcc ctc aag
```

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| Lys | Leu 50 | Ala | Asp | Pro | Ile | Thr 55 | Ile | Thr | Asn | Gly | Asp 60 | Val | Ser | Leu | Lys | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-----|
| gtg Val 65 | gga Gly | ggt Gly | ggt Gly | ctc Leu | act Thr 70 | ttg Leu | caa Gln | gat Asp | gga Gly | agc Ser 75 | cta Leu | act Thr | gta Val | aac Asn | cct Pro 80 | 240 |
| aag Lys | gct Ala | cca Pro | ctg Leu | caa Gln 85 | gtt Val | aat Asn | act Thr | gat Asp | aaa Lys 90 | aaa Lys | ctt Leu | gag Glu | ctt Leu | gca Ala 95 | tat Tyr | 288 |
| gat Asp | aat Asn | cca Pro | ttt Phe 100 | gaa Glu | agt Ser | agt Ser | gct Ala | aat Asn 105 | aaa Lys | ctt Leu | agt Ser | tta Leu | aáa Lys 110 | gta Val | gga Gly | 336 |
| cat His | gga Gly | tta Leu 115 | aaa Lys | gta Val | tta Leu | gat Asp | gaa Glu 120 | aaa Lys | agt Ser | gct Ala | gcg Ala | 999 Gly 125 | tta Leu | aaa Lys | gat Asp | 384 |
| tta Leu | att Ile 130 | ggc Gly | aaa Lys | ctt Leu | gtg Val | gtt Val 135 | tta Leu | aca Thr | gga Gly | aaa Lys | gga Gly 140 | ata Ile | ggc Gly | act Thr | gaa Glu | 432 |
| aat Asn 145 | tta Leu | gaa Glu | aat Asn | aca Thr | gat Asp 150 | ggt Gly | agc Ser | agc Ser | aga Arg | gga Gly 155 | att Ile | ggt Gly | ata Ile | aat Asn | gta Val 160 | 480 |
| | gca Ala | | | | | | | | | | | | | | | 528 |
| | aac Asn | | | | | | | | | | | | | | | 576 |
| | cca Pro | | | | | | | | | | | | | | | 624 |
| | ctt Leu 210 | | | | | | | | | | | | | | | 672 |
| | gtc Val | | | | | | | | | | | | | | | 720 |
| ata Ile | aaa Lys | agt Ser | ttt Phe | act Thr 245 | att Ile | aaa Lys | ctg Leu | cta Leu | ttt Phe 250 | aat Asn | aag Lys | aac Asn | gga Gly | gtg Val 255 | ctt Leu | 768 |
| tta Leu | gac Asp | aac Asn | tca Ser 260 | aat Asn | ctt Leu | gga Gly | aaa Lys | gct Ala 265 | tat Tyr | tgg Trp | aac Asn | ttt Phe | aga Arg 270 | agt Ser | gga Gly | 816 |
| | tcc Ser | | | | | | | | | | | | | | | 864 |
| aat Asn | ttg Leu | gta Val | gcg Ala | tat Tyr | cca Pro | aaa Lys | ccc Pro | agt Ser | aat Asn | tct Ser | aaa Lys | aaa Lys | tat Tyr | gca Ala | aga Arg | 912 |

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| 290 | | | | | 295 | | | | | 300 | | | | | |
|---------------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------|
| gac ata Asp Ile 305 | | | | | | | | | | | | | | | 960 |
| gca gtc Ala Val | | | | | | | | | | | | | | | 1008 |
| atc aca Ile Thr | | | | | | | | | | | | | | | 1056 |
| gaa acc Glu Thr | | | | | | | | | | | | | | | 1098 |
| <210> 3 <211> 3 <212> P <213> A | 65 RT | virus | s tyl | pe 31 | 7 | | | | | | | | | | |
| <400> 3 Met Ser | | Δrα | T.011 | Δrα | T = T | Glu | Zan | Zan | Dhe | Δen | Dro | Tall | ידי, | Pro | |
| 1 | _ | _ | 5 | _ | | | _ | 10 | | | | | 15 | | |
| Tyr Gly | _ | 20 | | | | | 25 | | | | | 30 | | | |
| Val Ser | Ser 35 | Asp | Gly | Phe | Lys | Asn 40 | Phe | Pro | Pro | Gly | Val 45 | Leu | Ser | Leu | |
| Lys Leu | Ala | Asp | Pro | Ile | Thr 55 | Ile | Thr | Asn | Gly | Asp 60 | Val | Ser | Leu | Lys | |
| Val Gly 65 | Gly | Gly | Leu | Thr 70 | | Gln | Asp | Gly | Ser 75 | | Thr | Val | Asn | Pro 80 | |
| Lys Ala | Pro | Leu | Gln 85 | | Asn | Thr | Asp | Lys 90 | | Leu | Glu | Leu | Ala 95 | | |
| Asp Asn | Pro | | | Ser | Ser | Ala | | | Leu | Ser | Leu | Lys 110 | | Gly | |
| His Gly | | 100 Lys | Val | Leu | Asp | | 105 Lys | Ser | Ala | Ala | | | Lys | Asp | |
| Leu Ile | 115 Gly | Lys | Leu | Val | | 120 Leu | Thr | Gly | Lys | | 125 Ile | Gly | Thr | Glu | |
| 130 Asn Leu | Glu | Asn | Thr | | 135 Gly | Ser | Ser | Arg | | 140 Ile | Gly | Ile | Asn | | |
| 145 Arg Ala | Arg | Glu | | 150 Leu | Thr | Phe | Asp | | 155 Asp | Gly | Tyr | Leu | | 160 Ala | |
| Trp Asn | Pro | | 165 Tyr | Asp | Thr | Arg | | 170 Leu | Trp | Thr | Thr | | | Thr | |
| Ser Pro | | 180 Cys | Thr | Ile | Ala | | 185 Asp | Lys | Asp | Ser | | 190 Leu | | Leu | |
| Val Leu | | Lys | Cys | Gly | | 200 Gln | Ile | Leu | Ala | | 205 Val | Ser | Leu | Ile | |
| 210 Val Val | | Gly | Lys | | 215 His | Ile | Ile | Asn | | 220 Lys | Thr | Asn | Pro | | |
| 225 Ile Lys | Ser | Phe | | 230 Ile | Lys | Leu | Leu | | 235 Asn | Lys | Asn | Gly | | 240 Leu | |
| Leu Asp | Asn | Ser | 245 Asn | Leu | Gly | Lys | Ala | 250 Tyr | Trp | Asn | Phe | | 255 Ser | Gly | |
| Asn Ser | Asn | 260 Val | Ser | Thr | Ala | Tyr | 265 Glu | Lys | Ala | Ile | Gly | 270 Phe | Met | Pro | |
| | | | | | | | | | | | | | | | |

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| Asn | Leu | 275 Val | Ala | Tyr | Pro | Lys | 280 Pro | Ser | Asn | Ser | Lys | 285 Lys | Tyr | Ala | Arg | |
|------------------|----------------------------------|-------------------|-------------------|------------------|------------------|-------------------|-------------------|-------------------|------------------|------------------|-------------------|-------------------|-------------------|------------------|------------------|-----|
| Asp | 290 Ile | Val | Tyr | Gly | Thr | 295 Ile | Tyr | Leu | Gly | Gly | 300 Lys | Pro | Asp | Gln | Pro | |
| 305 Ala | Val | Ile | Lys | Thr | 310 Thr | Phe | Asn | Gln | | 315 Thr | Gly | Cys | Glu | | 320 Ser | |
| Ile | Thr | Phe | | 325 Phe | Ser | Trp | Ser | | 330 Thr | Tyr | Glu | Asn | | 335 Glu | Phe | |
| Glu | Thr | Thr 355 | 340 Ser | Phe | Thr | Phe | Ser 360 | 345 Tyr | Ile | Ala | Gln | Glu 365 | 350 | | | |
| <213 <212 | 0> 33 L> 10 2> Di 3> Ad | 98 NA | virus | s tyr | oe 19 |)p | | | | | | | | | | |
| | L> CI | os 1) | .(109 | 98) | | | | | | | | | | | | |
| ata | 0> 33 tca Ser | aaq | agg Arg | ctc Leu 5 | cgg Arg | gtg Val | gaa Glu | gat Asp | gac Asp 10 | ttc Phe | aac Asn | ccc Pro | gtc Val | tac Tyr 15 | ccc Pro | 48 |
| tat Tyr | ggc Gly | tac Tyr | gcg Ala 20 | cgg Arg | aat Asn | cag Gln | aat Asn | atc Ile 25 | ccc Pro | ttc Phe | ctc Leu | act Thr | ccc Pro 30 | ccc Pro | ttt Phe | 96 |
| gtc Val | tcc Ser | tcc Ser 35 | gat Asp | gga Gly | ttc Phe | aaa Lys | aac Asn 40 | ttc Phe | ccc Pro | cct Pro | gly aaa | gta Val 45 | ctg Leu | tca Ser | ctc Leu | 144 |
| aaa Lys | ctg Leu 50 | gct Ala | gat Asp | cca Pro | atc Ile | acc Thr 55 | att Ile | acc Thr | aat .Asn | gly aaa | gat Asp 60 | gta Val | tcc Ser | ctc Leu | aag Lys | 192 |
| gtg Val 65 | Gly | ggt Gly | ggt Gly | ctc Leu | act Thr 70 | ttg Leu | caa Gln | gat Asp | gga Gly | agc Ser 75 | Leu | act Thr | gta Val | aac Asn | cct Pro 80 | 240 |
| aag Lys | gct Ala | cca Pro | ctg Leu | caa Gln 85 | gtt Val | act Thr | act Thr | gat Asp | aaa Lys 90 | Lys | ctt Leu | gag Glu | ctt Leu | gca Ala 95 | tat Tyr | 288 |
| gat Asp | aat Asn | cca Pro | ttt Phe 100 | gaa Glu | tgt Cys | agt Ser | gct Ala | aat Asn 105 | Lys | ttt Phe | agt Ser | tta Leu | aaa Lys 110 | gta Val | gga Gly | 336 |
| cat His | gga Gly | tta Leu 115 | Lys | gta Val | tta Leu | gat Asp | gaa Glu 120 | Lys | agt Ser | gct Ala | gcg Ala | 999 Gly 125 | Leu | aaa Lys | gat Asp | 384 |
| tta Leu | att Ile 130 | Gly | aaa Lys | ctt Leu | gtg Val | gtt Val 135 | Leu | aca Thr | gga Gly | aaa Lys | gga Gly 140 | тте | ggc Gly | act Thr | gaa Glu | 432 |
| aat Asn | tta Leu | gaa Glu | aat Asn | aca Thr | gat Asp | ggt Gly | ago Ser | ago Ser | aga Arg | gga Gly | att Ile | ggt Gly | ata Ile | aat Asn | gta Val | 480 |

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| 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| aga Arg | gca Ala | aga Arg | gaa Glu | 999 Gly 165 | ttg Leu | aca Thr | ttt Phe | gac Asp | aat Asn 170 | gat Asp | gga Gly | tac Tyr | ttg Leu | gta Val 175 | gca Ala | 528 |
| tgg Trp | aac Asn | cca Pro | aag Lys 180 | tat Tyr | gac Asp | acg Thr | cgc Arg | aca Thr 185 | ctt Leu | tgg Trp | aca Thr | aca Thr | cca Pro 190 | gac Asp | aca Thr | 576 |
| tct Ser | cca Pro | aac Asn 195 | tgc Cys | aca Thr | att Ile | gct Ala | cag Gln 200 | gat Asp | aag Lys | gac Asp | tct Ser | aaa Lys 205 | ctc Leu | act Thr | ttg Leu | 624 |
| gta Val | ctt Leu 210 | aca Thr | aag Lys | tgt Cys | gga Gly | agt Ser 215 | caa Gln | ata Ile | tta Leu | gct Ala | aat Asn 220 | gtg Val | tct Ser | ttg Leu | att Ile | 672 |
| gtg Val 225 | gtc Val | gca Ala | gga Gly | aag Lys | tac Tyr 230 | cac His | atc Ile | ata Ile | aat Asn | aat Asn 235 | aag Lys | aca Thr | aat Asn | cca Pro | gaa Glu 240 | 720 |
| ata Ile | aaa Lys | agt Ser | ttt Phe | act Thr 245 | att Ile | aaa Lys | ctg Leu | tta Leu | ttt Phe 250 | aat Asn | aag Lys | aac Asn | gga Gly | gtg Val 255 | ctt Leu | 768 |
| tta Leu | gac Asp | aac Asn | tca Ser 260 | aat Asn | ctt Leu | gga Gly | aaa Lys | gct Ala 265 | tat Tyr | tgg Trp | aac Asn | ttt Phe | aga Arg 270 | agt Ser | gga Gly | 816 |
| aat Asn | tcc Ser | aat Asn 275 | gtt Val | tcg Ser | aca Thr | gct Ala | tat Tyr 280 | gaa Glu | aaa Lys | gca Ala | att Ile | ggt Gly 285 | ttt Phe | atg Met | cct Pro | 864 |
| aat Asn | tta Leu 290 | gta Val | gcg Ala | tat Tyr | cca Pro | aaa Lys 295 | ccc Pro | agt Ser | aat Asn | tct Ser | aaa Lys 300 | aaa Lys | tat Tyr | gca Ala | aga Arg | 912 |
| gac Asp 305 | ata Ile | gtt Val | tat Tyr | gga Gly | act Thr 310 | ata Ile | tat Tyr | ctt Leu | ggt Gly | gga Gly 315 | aaa Lys | cct Pro | gat Asp | cag Gln | cca Pro 320 | 960 |
| gca Ala | gtc Val | att Ile | aaa Lys | act Thr 325 | acc Thr | ttt Phe | aac Asn | caa Gln | gaa Glu 330 | act Thr | gga Gly | tgt Cys | gaa Glu | tac Tyr 335 | tct Ser | 1008 |
| atc Ile | aca Thr | ttt Phe | gac Asp 340 | ttt Phe | agt Ser | tgg Trp | tcc Ser | aaa Lys 345 | acc Thr | tat Tyr | gaa Glu | aat Asn | gtt Val 350 | gaa Glu | ttt Phe | 1056 |
| gaa Glu | acc Thr | acc Thr 355 | tct Ser | ttt Phe | acc Thr | ttc Phe | tcc Ser 360 | Tyr | att Ile | gcc Ala | caa Gln | gaa Glu 365 | tga * | | | 1098 |

<210> 34 <211> 365 <212> PRT <213> Adenovirus type 19p

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Met Ser Lys Arg Leu Arg Val Glu Asp Asp Phe Asn Pro Val Tyr Pro
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Tyr Gly Tyr Ala Arg Asn Gln Asn Ile Pro Phe Leu Thr Pro Pro Phe
                               25
Val Ser Ser Asp Gly Phe Lys Asn Phe Pro Pro Gly Val Leu Ser Leu
 35
                          40
Lys Leu Ala Asp Pro Ile Thr Ile Thr Asn Gly Asp Val Ser Leu Lys
                      55
Val Gly Gly Gly Leu Thr Leu Gln Asp Gly Ser Leu Thr Val Asn Pro
                   70
                                       75
Lys Ala Pro Leu Gln Val Thr Thr Asp Lys Leu Glu Leu Ala Tyr
                                 90
Asp Asn Pro Phe Glu Cys Ser Ala Asn Lys Phe Ser Leu Lys Val Gly
                                105
His Gly Leu Lys Val Leu Asp Glu Lys Ser Ala Ala Gly Leu Lys Asp
                           120
Leu Ile Gly Lys Leu Val Val Leu Thr Gly Lys Gly Ile Gly Thr Glu
                    135
                                           140
Asn Leu Glu Asn Thr Asp Gly Ser Ser Arg Gly Ile Gly Ile Asn Val
145 150 155 160
Arg Ala Arg Glu Gly Leu Thr Phe Asp Asn Asp Gly Tyr Leu Val Ala
                                  170 175
               165
Trp Asn Pro Lys Tyr Asp Thr Arg Thr Leu Trp Thr Thr Pro Asp Thr
                                185
            180
Ser Pro Asn Cys Thr Ile Ala Gln Asp Lys Asp Ser Lys Leu Thr Leu
                          200
                                           205
Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Asn Val Ser Leu Ile
                        215
                                            220
Val Val Ala Gly Lys Tyr His Ile Ile Asn Asn Lys Thr Asn Pro Glu
225 230 235 240
Ile Lys Ser Phe Thr Ile Lys Leu Leu Phe Asn Lys Asn Gly Val Leu
               245
                                    250
Leu Asp Asn Ser Asn Leu Gly Lys Ala Tyr Trp Asn Phe Arg Ser Gly
                              265
            260
Asn Ser Asn Val Ser Thr Ala Tyr Glu Lys Ala Ile Gly Phe Met Pro
                                                285
                            280
Asn Leu Val Ala Tyr Pro Lys Pro Ser Asn Ser Lys Lys Tyr Ala Arg
                                            300
                        295
Asp Ile Val Tyr Gly Thr Ile Tyr Leu Gly Gly Lys Pro Asp Gln Pro
                    310
                                        315
Ala Val Ile Lys Thr Thr Phe Asn Gln Glu Thr Gly Cys Glu Tyr Ser
                325
                                    330
Ile Thr Phe Asp Phe Ser Trp Ser Lys Thr Tyr Glu Asn Val Glu Phe
          340 345
Glu Thr Thr Ser Phe Thr Phe Ser Tyr Ile Ala Gln Glu
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<211> 1116
<212> DNA
<213> Adenovirus type 30
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<221> CDS
<222> (1) ... (1116)
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Met Ser Lys Arg Leu Arg Val Glu Asp Asp Phe Asn Pro Val Tyr Pro
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<400> 34

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| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
|-------------------|-------------------|------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------------|-------------------|-------------------|-------------------|-------------------|-----|
| tat Tyr | ggc Gly | tac Tyr | gcg Ala 20 | cgg Arg | aat Asn | cag Gln | aat Asn | atc Ile 25 | ccc Pro | ttc Phe | ctt Leu | act Thr | ccc Pro 30 | ccc Pro | ttt Phe | 96 |
| gtc Val | tca Ser | tcc Ser 35 | gat Asp | gga Gly | ttc Phe | aaa Lys | aac Asn 40 | ttc Phe | cca Pro | cct Pro | gj ^j aaa | gtc Val 45 | ctg Leu | tca Ser | ctc Leu | 144 |
| aaa Lys | ctg Leu 50 | gct Ala | gac Asp | cca Pro | atc Ile | gcc Ala 55 | atc Ile | act Thr | aat Asn | Gly aaa | gat Asp 60 | gtc Val | tca Ser | ctc Leu | aag Lys | 192 |
| gtg Val 65 | gga Gly | gl ^à aaa | gga Gly | cta Leu | act Thr 70 | gtg Val | gaa Glu | caa Gln | gat Asp | agt Ser 75 | gga Gly | aac Asn | cta Leu | agt Ser | gta Val 80 | 240 |
| aac Asn | cct Pro | aag Lys | gct Ala | cca Pro 85 | ttg Leu | caa Gln | gtt Val | gga Gly | aca Thr 90 | gac Asp | aaa Lys | aaa Lys | ctg Leu | gaa Glu 95 | ttg Leu | 288 |
| gct Ala | tta Leu | gca Ala | cct Pro 100 | cca Pro | ttt Phe | gat Asp | gtc Val | aga Arg 105 | gat Asp | aac Asn | aag Lys | cta Leu | gct Ala 110 | att Ile | cta Leu | 336 |
| gta Val | gga Gly | gat Asp 115 | gga Gly | tta Leu | aag Lys | gta Val | ata Ile 120 | gat Asp | aga Arg | tca Ser | ata Ile | tct Ser 125 | gat Asp | ttg Leu | cca Pro | 384 |
| | | | | | | | | ttg Leu | | | | | | | | 432 |
| | | | | | | | | agc Ser | | | | | | | | 480 |
| gtg Val | aga Arg | att Ile | gga Gly | gaa Glu 165 | gga Gly | ggt Gly | ggt Gly | tta Leu | act Thr 170 | ttt Phe | gat Asp | gat Asp | aaa Lys | ggt Gly 175 | tat Tyr | 528 |
| | | | | | | | | gac Asp 185 | | | | | | | | 576 |
| tta Leu | gac Asp | cct Pro 195 | tct Ser | cca Pro | aat Asn | tgt Cys | aag Lys 200 | ata Ile | gat Asp | ata Ile | gaa Glu | aaa Lys 205 | gac Asp | tca Ser | aaa Lys | 624 |
| cta Leu | act Thr 210 | ttg Leu | gta Val | ctg Leu | aca Thr | aag Lys 215 | tgc Cys | gga Gly | agt Ser | cag Gln | att Ile 220 | ttg Leu | gca Ala | aat Asn | gta Val | 672 |
| tct Ser 225 | cta Leu | att Ile | ata Ile | gtc Val | aac Asn 230 | gga Gly | aag Lys | ttc Phe | aag Lys | atc Ile 235 | ctt Leu | aat Asn | aac Asn | aaa Lys | aca Thr 240 | 720 |
| | | | | | | | | aac Asn | | | | | | | | 768 |

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| aat Asn | gga Gly | gtt Val | cta Leu 260 | ttg Leu | gaa Glu | aat Asn | tca Ser | aac Asn 265 | att Ile | gaa Glu | aaa Lys | cag Gln | tac Tyr 270 | cta Leu | aac Asn | 816 |
|---|--|--|--|--|---|--|--|--|---|---|--|--|--|---|---|------|
| ttt Phe | aga Arg | agt Ser 275 | gga Gly | gac Asp | tct Ser | att Ile | ctt Leu 280 | cca Pro | gag Glu | cca Pro | tat Tyr | aaa Lys 285 | aat Asn | gca Ala | att Ile | 864 |
| gga Gly | ttt Phe 290 | atg Met | cct Pro | aat Asn | tta Leu | cta Leu 295 | gct Ala | tat Tyr | gct Ala | aaa Lys | gct Ala 300 | aca Thr | act Thr | gat Asp | cag Gln | 912 |
| tct Ser 305 | aaa Lys | att Ile | tat Tyr | gca Ala | agg Arg 310 | aac Asn | act Thr | ata Ile | tat Tyr | gga Gly 315 | aat Asn | atc Ile | tac Tyr | tta Leu | gat Asp 320 | 960 |
| aat Asn | cag Gln | cca Pro | tat Tyr | aat Asn 325 | cca Pro | gtt Val | gta Val | att Ile | aaa Lys 330 | att Ile | act Thr | ttt Phe | aat Asn | aat Asn 335 | gaa Glu | 1008 |
| gca Ala | gat Asp | agt Ser | gct Ala 340 | tat Tyr | tct Ser | atc Ile | act Thr | ttt Phe 345 | aac Asn | tat Tyr | tca Ser | tgg Trp | acc Thr 350 | aag Lys | gac Asp | 1056 |
| tat Tyr | gac Asp | aat Asn 355 | atc Ile | cct Pro | ttt Phe | gat Asp | tct Ser 360 | act Thr | tca Ser | ttt Phe | acc Thr | ttc Phe 365 | tcc Ser | tat Tyr | atc Ile | 1104 |
| gcc | caa | | _ | | | | | | | | | | | | | 1116 |
| Ala | Gln 370 | Glu | * | | | | | | | | | | | | | |
| <210 <213 <213 | 370 D> 36 L> 37 Z> PF | 5 70 RT | * rirus | s tyr | oe 30 |) | | | | | | | | | | |
| <210 <213 <213 <400 Ser | 370 D> 36 L> 37 2> PF B> A6 | 5 70 RT denov | <i>r</i> irus | Arg | | | Asp | Asp | | Asn | Pro | Val | Tyr | Pro | Tyr | |
| <210 <211 <212 <213 <400 Ser 1 | 370 0> 36 L> 37 2> PF 3> Ad 0> 36 Lys | 5 70 RT denov Arg | virus Leu Arg | Arg 5 | Val | Glu | | Pro | 10 | | Pro Thr | | Pro | 15 | - | |
| <210 <211 <212 <213 <400 Ser 1 Gly | 370 0> 36 L> 37 2> PF 3> Ac 0> 36 Lys | 5 70 RT denov Arg Ala Asp | rirus Leu Arg 20 | Arg 5 Asn | Val Gln | Glu Asn | Ile Phe | Pro 25 | 10 Phe | Leu | | Pro Leu | Pro 30 | 15 Phe | Val | |
| <210 <211 <212 <213 <400 Ser 1 Gly Ser | 370)> 36 L> 37 2> PF 3> Ac Lys Tyr Ser Ala | 5 70 RT denov 5 Arg Ala Asp 35 | virus Leu Arg 20 Gly | Arg 5 Asn Phe | Val Gln Lys | Glu Asn Asn Ile | Ile Phe 40 | Pro 25 Pro | 10 Phe Pro | Leu Gly | Thr Val Val | Pro Leu 45 | Pro 30 Ser | 15 Phe Leu | Val Lys | |
| <210 <211 <211 <213 <400 Ser 1 Gly Ser Leu | 370)> 36 L> 37 3> AG Lys Tyr Ser Ala 50 | 6 70 RT denov Arg Ala Asp 35 Asp | virus Leu Arg 20 Gly Pro | Arg 5 Asn Phe Ile | Val Gln Lys Ala | Glu Asn Asn Ile 55 | Ile Phe 40 Thr | Pro 25 Pro Asn | 10 Phe Pro Gly | Leu Gly Asp | Thr Val | Pro Leu 45 Ser | Pro 30 Ser Leu | 15 Phe Leu Lys | Val Lys Val Asn | |
| <210 <211 <211 <213 <400 Ser 1 Gly Ser Leu Gly 65 | 370)> 36 L> 37 3> Ac Lys Tyr Ser Ala 50 Gly | 6 70 RT denov Arg Ala Asp 35 Asp | rirus Leu Arg 20 Gly Pro Leu | Arg 5 Asn Phe Ile Thr | Val Gln Lys Ala Val 70 | Glu Asn Asn Ile 55 Glu | Ile Phe 40 Thr Gln | Pro 25 Pro Asn Asp | 10 Phe Pro Gly Ser | Leu Gly Asp Gly 75 | Thr Val Val 60 | Pro Leu 45 Ser Leu | Pro 30 Ser Leu Ser | 15 Phe Leu Lys Val Leu | Val Lys Val Asn 80 | |
| <210 <211 <212 <213 <400 Ser 1 Gly Ser Leu Gly 65 Pro | 370)> 36 L> 37 2> PF 3> Ac Lys Tyr Ser Ala 50 Gly Lys Ala | 6 70 RT denov 6 Arg Ala Asp 35 Asp Gly Ala Pro | Leu Arg 20 Gly Pro Leu Pro 100 | Arg 5 Asn Phe Ile Thr Leu 85 Phe | Val Gln Lys Ala Val 70 Gln Asp | Glu Asn Asn Ile 55 Glu Val | Ile Phe 40 Thr Gln Gly Arg | Pro 25 Pro Asn Asp Thr Asp 105 | 10 Phe Pro Gly Ser Asp 90 Asn | Leu Gly Asp Gly 75 Lys | Thr Val Val 60 Asn Lys Leu | Pro Leu 45 Ser Leu Leu Ala | Pro 30 Ser Leu Ser Glu Ile 110 | 15 Phe Leu Lys Val Leu 95 Leu | Val Lys Val Asn 80 Ala | |
| <210 <211 <212 <213 <400 Ser 1 Gly Ser Leu Gly 65 Pro Leu Gly | 370)> 36 L> 37 2> PF 3> Ac Lys Tyr Ser Ala 50 Gly Lys Ala Asp | Arg Ala Asp 35 Asp Gly Ala Pro Gly 115 | Leu Arg 20 Gly Pro Leu Pro 100 Leu | Arg 5 Asn Phe Ile Thr Leu 85 Phe | Val Gln Lys Ala Val 70 Gln Asp | Glu Asn Asn Ile 55 Glu Val Val Ile | Ile Phe 40 Thr Gln Gly Arg Asp 120 | Pro 25 Pro Asn Asp Thr Asp 105 Arg | 10 Phe Pro Gly Ser Asp 90 Asn Ser | Leu Gly Asp Gly 75 Lys Lys | Thr Val Val 60 Asn Lys Leu Ser | Pro Leu 45 Ser Leu Leu Ala Asp | Pro 30 Ser Leu Ser Glu Ile 110 Leu | 15 Phe Leu Lys Val Leu 95 Leu | Val Lys Val Asn 80 Ala Val | |
| <210<211<212<213<400 Ser 1 Gly Ser Leu Gly 65 Pro Leu Gly Leu Leu | 370)> 36 L> 37 2> PF 3> Ac Lys Tyr Ser Ala 50 Gly Lys Ala Asp Leu 130 | Arg Ala Asp 35 Asp Gly Ala Pro Gly 115 Asn | Leu Arg 20 Gly Pro Leu Pro 100 Leu Tyr | Arg 5 Asn Phe Ile Thr Leu 85 Phe Lys Leu | Val Gln Lys Ala Val 70 Gln Asp Val Val | Glu Asn Asn Ile 55 Glu Val Val Ile Val 135 | Phe 40 Thr Gln Gly Arg Asp 120 Leu | Pro 25 Pro Asn Asp Thr Asp 105 Arg | 10 Phe Pro Gly Ser Asp 90 Asn Ser Gly | Leu Gly Asp Gly 75 Lys Lys Ile | Thr Val Val 60 Asn Lys Leu Ser Gly 140 | Pro Leu 45 Ser Leu Leu Ala Asp 125 Ile | Pro 30 Ser Leu Ser Glu Ile 110 Leu | 15 Phe Leu Lys Val Leu 95 Leu Pro Asn | Val Lys Val Asn 80 Ala Val Gly | |
| <210<211<211<211<211<211<211<211<211<211 | 370)> 36 L> 37 2> PF 3> Ac Lys Tyr Ser Ala 50 Gly Lys Ala Asp Leu 130 Leu | Arg Ala Asp 35 Asp Gly Ala Pro Gly 115 Asn Lys | Leu Arg 20 Gly Pro Leu Pro 100 Leu Tyr Asn | Arg 5 Asn Phe Ile Thr Leu 85 Phe Lys Leu Asp | Val Gln Lys Ala Val 70 Gln Asp Val Asp | Glu Asn Ile 55 Glu Val Val Ile Val Ile Cal Ile | Phe 40 Thr Gln Gly Arg Asp 120 Leu Ser | Pro 25 Pro Asn Asp Thr Asp 105 Arg Thr Asn | 10 Phe Pro Gly Ser Asp 90 Asn Ser Gly Lys | Leu Gly Asp Gly 75 Lys Lys Lys Gly Gly 155 | Thr Val Val 60 Asn Lys Leu Ser Gly | Pro Leu 45 Ser Leu Leu Ala Asp 125 Ile Gly | Pro 30 Ser Leu Ser Glu Ile 110 Leu Gly Leu | 15 Phe Leu Lys Val Leu 95 Leu Pro Asn Cys | Val Lys Val Asn 80 Ala Val Gly Glu Val 160 | |

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| | | | | 165 | | | | | 170 | | | | | 175 | | |
|-----------------|------------------|------------|------------------|-----------------|------------|------------------|------------|------------------|------------|------------------|------------------|------------|------------------|------------------|------------------|-----|
| Val | Ala | Trp | Asn 180 | Asn | Lys | His | Asp | Ile 185 | Arg | Thr | Leu | Trp | Thr 190 | Thr | Leu | |
| Asp | Pro | Ser 195 | Pro | Asn | Cys | Lys | Ile 200 | Asp | Ile | Glu | Lys | Asp 205 | Ser | Lys | Leu | |
| Thr | Leu 210 | Val | Leu | Thr | Lys | Cys 215 | Gly | Ser | Gln | Ile | Leu 220 | Ala | Asn | Val | Ser | |
| Leu 225 | Ile | Ile | Val | Asn | Gly 230 | Lys | Phe | Lys | Ile | Leu 235 | Asn | Asn | Lys | Thr | Asp 240 | |
| | Ser | Leu | Pro | Lys 245 | Ser | Phe | Asn | Ile | Lys 250 | Leu | Leu | Phe | Asp | Gln 255 | Asn | |
| Gly | Val | Leu | Leu 260 | Glu | Asn | Ser | Asn | Ile 265 | Glu | Lys | Gln | Tyr | Leu 270 | Asn | Phe | |
| Arg | Ser | Gly 275 | | Ser | Ile | Leu | Pro 280 | Glu | Pro | Tyr | Lys | Asn 285 | Ala | Ile | Gly | |
| Phe | Met 290 | | Asn | Leu | Leu | Ala 295 | | Ala | Lys | Ala | Thr 300 | Thr | Asp | Gln | Ser | |
| Lys 305 | | Tyr | Ala | Arg | Asn 310 | Thr | Ile | Tyr | Gly | Asn 315 | Ile | Tyr | Leu | Asp | Asn 320 | |
| | Pro | Tyr | Asn | Pro 325 | | Val | Ile | Lys | Ile 330 | Thr | Phe | Asn | Asn | Glu 335 | Ala | |
| Asp | Ser | Ala | Tyr 340 | | Ile | Thr | Phe | Asn 345 | Tyr | Ser | Trp | Thr | Lys 350 | Asp | Tyr | |
| Asp | Asn | Ile 355 | Pro | Phe | Asp | Ser | Thr 360 | | Phe | Thr | Phe | Ser 365 | Tyr | Ile | Ala | |
| Gln | Glu 370 | | | | | | | | | | | | | | | |
| | 570 | | | | | | | | | | | | | | | |
| | 0 > 3 1 > 1 | | | | | | | | | | | | | | | |
| <21 | 2> D | | viru | s ty | pe 1 | б | | | | | | | | | | |
| | 1> C | DS 1) | .(10 | 62) | | | | | | | • | | | | | |
| <40 | 0 > 3 | 7 | | | | | | | | | 4_ | | | + ~~ | 999 | 48 |
| atg Met 1 | gcc Ala | aaa Lys | cga Arg | gct Ala 5 | cgg Arg | cta Leu | agc Ser | agc Ser | Ser 10 | Phe | aat Asn | Pro | Val | tac Tyr 15 | Pro | 40 |
| tat Tyr | gaa Glu | gat Asp | gaa Glu 20 | Ser | agc Ser | tca Ser | caa Gln | cac His 25 | ccc Pro | ttt Phe | ata Ile | aac Asn | cct Pro 30 | ggt Gly | ttc Phe | 96 |
| att | tco | tca | aat | aat. | ttt | gca | . caa | agc | cca | gat | gga | gtt | cta | act | ctt | 144 |
| Il€ | e Ser | Ser 35 | Asn | ĞĬy | Phe | Ăla | Gln 40 | Ser | Pro | Asp | Gly | Val 45 | Leu | . Thr | Leu | |
| aaa Lys | tgt Cys 50 | Val | aat Asn | cca Pro | ctc Leu | act Thr 55 | Thr | gcc Ala | agc Ser | gga Gly | ccc Pro 60 | Leu | caa Gln | ctt Leu | aaa Lys | 192 |
| gtt Val | . Gly | agc Ser | agt Ser | ctt Leu | aca Thr | · Val | gat Asp | act Thr | atc Ile | gat Asp 75 | GLy | tct Ser | ttg Leu | gag Glu | gaa Glu 80 | 240 |
| aat | | | | | | | | | | | | | | | | |

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| | | | | | | | | | | | | | | | • | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| | tta Leu | | | | | | | | | | | | | | | 336 |
| | ctg Leu | | | | | | | | | | | | | | | 384 |
| | gga Gly 130 | | | | | | | | | | | | | | | 432 |
| gga Gly 145 | cat His | ggc Gly | ctt Leu | gtg Val | ttt Phe 150 | gac Asp | tct Ser | tcc Ser | aat Asn | gct Ala 155 | atc Ile | acc Thr | ata Ile | gaa Glu | aac Asn 160 | 480 |
| aac Asn | acc Thr | ttg Leu | tgg Trp | aca Thr 165 | ggc Gly | gca Ala | aaa Lys | cca Pro | agc Ser 170 | gcc Ala | aac Asn | tgt Cys | gta Val | att Ile 175 | aaa Lys | 528 |
| gag Glu | gga Gly | gaa Glu | gat Asp 180 | tcc Ser | cca Pro | gac Asp | tgt Cys | aag Lys 185 | ctc Leu | act Thr | tta Leu | gtt Val | cta Leu 190 | gtg Val | aag Lys | 576 |
| aat Asn | gga Gly | gga Gly 195 | ctg Leu | ata Ile | aat Asn | gga Gly | tac Tyr 200 | ata Ile | aca Thr | tta Leu | atg Met | gga Gly 205 | gcc Ala | tca Ser | gaa Glu | 624 |
| tat Tyr | act Thr 210 | aac Asn | acc Thr | ttg Leu | ttt Phe | aaa Lys 215 | aac Asn | aat Asn | caa Gln | gtt Val | aca Thr 220 | atc Ile | gat Asp | gta Val | aac Asn | 672 |
| ctc Leu 225 | gca Ala | ttt Phe | gat Asp | aat Asn | act Thr 230 | ggc ggc | caa Gln | att Ile | att Ile | act Thr 235 | tac Tyr | cta Leu | tca Ser | tcc Ser | ctt Leu 240 | 720 |
| aaa Lys | agt Ser | aac Asn | ctg Leu | aac Asn 245 | ttt Phe | aaa Lys | gac Asp | aac Asn | caa Gln 250 | aac Asn | atg Met | gct Ala | act Thr | gga Gly 255 | acc Thr | 768 |
| ata Ile | acc Thr | agt Ser | gcc Ala 260 | aaa Lys | ggc Gly | ttc Phe | atg Met | ccc Pro 265 | agc Ser | acc Thr | acc Thr | gcc Ala | tat Tyr 270 | cca Pro | ttt Phe | 816 |
| ata Ile | aca Thr | tac Tyr 275 | gcc Ala | act Thr | gag Glu | acc Thr | cta Leu 280 | aat Asn | gaa Glu | gat Asp | tac Tyr | att Ile 285 | tat Tyr | gga Gly | gag Glu | 864 |
| tgt Cys | tac Tyr 290 | tac Tyr | aaa Lys | tct Ser | acc Thr | aat Asn 295 | gga Gly | act Thr | ctc Leu | ttt Phe | cca Pro 300 | cta Leu | aaa Lys | gtt Val | act Thr | 912 |
| gtc Val 305 | aca Thr | cta Leu | aac Asn | aga Arg | cgt Arg 310 | atg Met | tta Leu | gct Ala | tct Ser | gga Gly 315 | Met | gcc Ala | tat Tyr | gct Ala | atg Met 320 | 960 |
| aat Asn | ttt Phe | tca Ser | tgg Trp | tct Ser 325 | cta Leu | aat Asn | gca Ala | gag Glu | gaa Glu 330 | Ala | ccg Pro | gaa Glu | act Thr | acc Thr 335 | gaa Glu | 1008 |

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1056 $ar{ extsf{Val}}$ Thr Leu Ile Thr Ser Pro Phe Phe Phe Ser Tyr Ile Arg Glu Asp 345 1062 gac tga Asp * <210> 38 <211> 353 <212> PRT <213> Adenovirus type 16 <400> 38 Met Ala Lys Arg Ala Arg Leu Ser Ser Ser Phe Asn Pro Val Tyr Pro 10 Tyr Glu Asp Glu Ser Ser Ser Gln His Pro Phe Ile Asn Pro Gly Phe Ile Ser Ser Asn Gly Phe Ala Gln Ser Pro Asp Gly Val Leu Thr Leu 45 35 40 Lys Cys Val Asn Pro Leu Thr Thr Ala Ser Gly Pro Leu Gln Leu Lys 50 55 Val Gly Ser Ser Leu Thr Val Asp Thr Ile Asp Gly Ser Leu Glu Glu 75 70 Asn Ile Thr Ala Ala Ala Pro Leu Thr Lys Thr Asn His Ser Ile Gly 85 90 Leu Leu Ile Gly Ser Gly Leu Gln Thr Lys Asp Asp Lys Leu Cys Leu 105 Ser Leu Gly Asp Gly Leu Val Thr Lys Asp Asp Lys Leu Cys Leu Ser 120 115 Leu Gly Asp Gly Leu Ile Thr Lys Asn Asp Val Leu Cys Ala Lys Leu 135 140 Gly His Gly Leu Val Phe Asp Ser Ser Asn Ala Ile Thr Ile Glu Asn 155 150 Asn Thr Leu Trp Thr Gly Ala Lys Pro Ser Ala Asn Cys Val Ile Lys -170 165 Glu Gly Glu Asp Ser Pro Asp Cys Lys Leu Thr Leu Val Leu Val Lys 185 Asn Gly Gly Leu Ile Asn Gly Tyr Ile Thr Leu Met Gly Ala Ser Glu 205 200 Tyr Thr Asn Thr Leu Phe Lys Asn Asn Gln Val Thr Ile Asp Val Asn 220 215 Leu Ala Phe Asp Asn Thr Gly Gln Ile Ile Thr Tyr Leu Ser Ser Leu 235 230 Lys Ser Asn Leu Asn Phe Lys Asp Asn Gln Asn Met Ala Thr Gly Thr 250 255 245 Ile Thr Ser Ala Lys Gly Phe Met Pro Ser Thr Thr Ala Tyr Pro Phe 265 270 Ile Thr Tyr Ala Thr Glu Thr Leu Asn Glu Asp Tyr Ile Tyr Gly Glu 280 275 Cys Tyr Tyr Lys Ser Thr Asn Gly Thr Leu Phe Pro Leu Lys Val Thr 295 . 300 Val Thr Leu Asn Arg Arg Met Leu Ala Ser Gly Met Ala Tyr Ala Met 305 310 315 320 310 Asn Phe Ser Trp Ser Leu Asn Ala Glu Glu Ala Pro Glu Thr Thr Glu 325 330 Val Thr Leu Ile Thr Ser Pro Phe Phe Phe Ser Tyr Ile Arg Glu Asp 345 Asp

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<210> 39 <211> 972 <212> DNA <213> Adenovirus type 35 <220> <221> CDS <222> (1) ... (972) <400> 39 atg acc aag aga gtc cgg ctc agt gac tcc ttc aac cct gtc tac ccc 48 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt 96 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe 144 att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa 192 Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys gtg gga ggg gga ctt aca gtg gat gac act gat ggt acc tta caa gaa Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu 240 aac ata cgt gct aca gca ccc att act aaa aat aat cac tct gta gaa 288 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu cta tcc att gga aat gga tta gaa act caa aac aat aaa cta tgt gcc 336 Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala aaa ttg gga aat ggg tta aaa ttt aac aac ggt gac att tgt ata aag 384 Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys gat agt att aac acc tta tgg act gga ata aac cct cca cct aac tgt Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys caa att gtg gaa aac act aat aca aat gat ggc aaa ctt act tta gta 480 Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val 150 tta gta aaa aat gga ggg ctt gtt aat ggc tac gtg tct cta gtt ggt Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly 528 170 165 gta tca gac act gtg aac caa atg ttc aca caa aag aca gca aac atc Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile 185

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| caa Gln | tta Leu | aga Arg 195 | tta Leu | tat Tyr | ttt Phe | gac Asp | tct Ser 200 | tct Ser | gga Gly | aat Asn | cta Leu | tta Leu 205 | act Thr | gag Glu | gaa Glu | 624 |
|--|-------------------|------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-----|
| tca Ser | gac Asp 210 | tta Leu | aaa Lys | att Ile | cca Pro | ctt Leu 215 | aaa Lys | aat Asn | aaa Lys | tct Ser | tct Ser 220 | aca Thr | gcg Ala | acc Thr | agt Ser | 672 |
| gaa Glu 225 | act Thr | gta Val | gcc Ala | agc Ser | agc Ser 230 | aaa Lys | gcc Ala | ttt Phe | atg Met | cca Pro 235 | agt Ser | act Thr | aca Thr | gct Ala | tat Tyr 240 | 720 |
| ccc Pro | ttc Phe | aac Asn | acc Thr | act Thr 245 | act Thr | agg Arg | gat Asp | agt Ser | gaa Glu 250 | aac Asn | tac Tyr | att Ile | cat His | gga Gly 255 | ata Ile | 768 |
| tgt Cys | tac Tyr | tac Tyr | atg Met 260 | act Thr | agt Ser | tat Tyr | gat Asp | aga Arg 265 | agt Ser | cta Leu | ttt Phe | ccc Pro | ttg Leu 270 | aac Asn | att Ile | 816 |
| tct Ser | ata Ile | atg Met 275 | cta Leu | aac Asn | agc Ser | cgt Arg | atg Met 280 | att Ile | tct Ser | tcc Ser | aat Asn | gtt Val 285 | gcc Ala | tat Tyr | gcc Ala | 864 |
| ata Ile | caa Gln 290 | ttt Phe | gaa Glu | tgg Trp | aat Asn | cta Leu 295 | aat Asn | gca Ala | agt Ser | gaa Glu | tct Ser 300 | cca Pro | gaa Glu | agc Ser | aac Asn | 912 |
| ata Ile 305 | gct Ala | acg Thr | ctg Leu | acc Thr | aca Thr 310 | taa Ser | ccc Pro | ttt Phe | ttc Phe | ttt Phe 315 | tct Ser | tac Tyr | att Ile | aca Thr | gaa Glu 320 | 960 |
| = | gac Asp | | taa * | | | | | | | | | | | | | 972 |
| <211 <212 <213 <400 Met 1 | | 3 T lenov Lys | Arg | Val 5 | Arg | Leu | | | 10 | | | | | 15 | Pro · | |
| Tyr | Glu | qaA | Glu 20 | | Thr | | Gln | | Pro | Phe | Ile | Asn | Pro 30 | Gly | Phe | |
| Ile | Ser | Pro 35 | | | Phe | | | | Pro | Asp | Gly | Val 45 | | Thr | Leu | |
| Lys | Cys 50 | | Thr | Pro | Leu | Thr 55 | | Thr | Gly | Gly | Ser 60 | | Gln | Leu | Lys | |
| Val 65 | | Gly | Gly | Leu | Thr 70 | | Asp | Asp | Thr | Asp | | Thr | Leu | Gln | Glu 80 | |
| Asn | Ile | Arg | Ala | Thr 85 | | Pro | Ile | Thr | Lys 90 | | Asn | His | Ser | Val 95 | Glu | |
| Leu | Ser | Ile | Gly 100 | | Gly | Leu | Glu | Thr 105 | | Asn | Asn | Lys | Leu 110 | Cys | Ala | |
| Lys | Leu | Gly 115 | Asn | Gly | Leu | Lys | Phe 120 | | Asn | Gly | Asp | Ile 125 | Cys | Ile | Lys | |
| Asp | Ser 130 | | Asn | Thr | Leu | Trp 135 | | Gly | Ile | Asn | Pro 140 | Pro | Pro | Asn | Cys | |

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Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val 155 150 Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly 165 170 Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile 185 Gln Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu 195 200 205 Ser Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser 215 220 Glu Thr Val Ala Ser Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr 235 230 Pro Phe Asn Thr Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile 250 245 Cys Tyr Tyr Met Thr Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile 265 270 Ser Ile Met Leu Asn Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala 275 280 285 Ile Gln Phe Glu Trp Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn 295 Ile Ala Thr Leu Thr Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu 310 . 315 Asp Asp Asn <210> 41 <211> 1062 <212> DNA <213> Artificial Sequence <220> <223> Ad5/Ad16 chimeric fiber <221> CDS <222> (1)...(1062) atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro 10 tat gaa gat gaa agc agc tca caa cac ccc ttt ata aac cct ggt ttc 96 Tyr Glu Asp Glu Ser Ser Ser Gln His Pro Phe Ile Asn Pro Gly Phe att tcc tca aat ggt ttt gca caa agc cca gat gga gtt cta act ctt Ile Ser Ser Asn Gly Phe Ala Gln Ser Pro Asp Gly Val Leu Thr Leu 40 aaa tgt gtt aat cca ctc act acc gcc agc gga ccc ctc caa ctt aaa Lys Cys Val Asn Pro Leu Thr Thr Ala Ser Gly Pro Leu Gln Leu Lys gtt gga agc agt ctt aca gta gat act atc gat ggg tct ttg gag gaa Val Gly Ser Ser Leu Thr Val Asp Thr Ile Asp Gly Ser Leu Glu Glu 240 65 aat ata act gcc gca gcg cca ctc act aaa act aac cac tcc ata ggt 288 Asn Ile Thr Ala Ala Ala Pro Leu Thr Lys Thr Asn His Ser Ile Gly 90 85

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| tta Leu | tta Leu | ata Ile | gga Gly 100 | tct Ser | gly | ttg Leu | caa Gln | aca Thr 105 | aag Lys | gat Asp | gat Asp | aaa Lys | ctt Leu 110 | tgt Cys | tta Leu | 336 |
|-------------------|-------------------|-------------------|------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| tcg Ser | ctg Leu | gga Gly 115 | gat Asp | Gly aaa | ttg Leu | gta Val | aca Thr 120 | aag Lys | gat Asp | gat Asp | aaa Lys | cta Leu 125 | tgt Cys | tta Leu | tcg Ser | 384 |
| ctg Leu | gga Gly 130 | gat Asp | gl ^à aaa | tta Leu | ata Ile | aca Thr 135 | aaa Lys | aat Asn | gat Asp | gta Val | cta Leu 140 | tgt Cys | gcc Ala | aaa Lys | cta Leu | 432 |
| gga Gly 145 | cat His | ggc Gly | ctt Leu | gtg Val | ttt Phe 150 | gac Asp | tct Ser | tcc Ser | aat Asn | gct Ala 155 | atc Ile | acc Thr | ata Ile | gaa Glu | aac Asn 160 | 480 |
| aac Asn | acc Thr | ttg Leu | tgg Trp | aca Thr 165 | gly ggc | gca Ala | aaa Lys | cca Pro | agc Ser 170 | gcc Ala | aac Asn | tgt Cys | gta Val | att Ile 175 | aaa Lys | 528 |
| gag Glu | gga Gly | gaa Glu | gat Asp 180 | tcc Ser | cca Pro | gac Asp | tgt Cys | aag Lys 185 | ctc Leu | act Thr | tta Leu | gtt Val | cta Leu 190 | gtg Val | aag Lys | 576 |
| aat Asn | gga Gly | gga Gly 195 | ctg Leu | ata Ile | aat Asn | gga Gly | tac Tyr 200 | ata Ile | aca Thr | tta Leu | atg Met | gga Gly 205 | gcc Ala | tca Ser | gaa Glu | 624 |
| tat Tyr | act Thr 210 | aac Asn | acc Thr | ttg Leu | ttt Phe | aaa Lys 215 | aac Asn | aat Asn | caa Gln | gtt Val | aca Thr 220 | atc Ile | gat Asp | gta Val | aac Asn | 672 |
| ctc Leu 225 | gca Ala | ttt Phe | gat Asp | aat Asn | act Thr 230 | ggc gly | caa Gln | att Ile | att Ile | act Thr 235 | tac Tyr | cta Leu | tca Ser | tcc Ser | ctt Leu 240 | 720 |
| aaa Lys | agt Ser | aac Asn | ctg Leu | aac Asn 245 | ttt Phe | aaa Lys | gac Asp | aac Asn | caa Gln 250 | aac Asn | atg Met | gct Ala | act Thr | gga Gly 255 | acc Thr | 768 |
| ata Ile | acc Thr | agt Ser | gcc Ala 260 | aaa Lys | gly ggc | ttc Phe | atg Met | ccc Pro 265 | agc Ser | acc Thr | acc Thr | gcc Ala | tat Tyr 270 | cca Pro | ttt Phe | 816 |
| ata Ile | aca Thr | tac Tyr 275 | gcc Ala | act Thr | gag Glu | acc Thr | cta Leu 280 | aat Asn | gaa Glu | gat Asp | tac Tyr | att Ile 285 | tat Tyr | gga Gly | gag Glu | 864 |
| | tac Tyr 290 | | | | | | | | | | | | | | | 912 |
| gtc Val 305 | aca Thr | cta Leu | aac Asn | aga Arg | cgt Arg 310 | atg Met | tta Leu | gct Ala | tct Ser | gga Gly 315 | atg Met | gcc Ala | tat Tyr | gct Ala | atg Met 320 | 960 |
| aat Asn | ttt Phe | tca Ser | tgg Trp | tct Ser 325 | cta Leu | aat Asn | gca Ala | gag Glu | gaa Glu 330 | gcc Ala | ccg Pro | gaa Glu | act Thr | acc Thr 335 | gaa Glu | 1008 |

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1056
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gac tga
                                                             1062
Asp *
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<211> 353
<212> PRT
<213> Artificial Sequence
<223> Ad5/Ad16 chimeric fiber
<400> 42
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
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                                10
Tyr Glu Asp Glu Ser Ser Ser Gln His Pro Phe Ile Asn Pro Gly Phe
                             25
Ile Ser Ser Asn Gly Phe Ala Gln Ser Pro Asp Gly Val Leu Thr Leu
                         40
                                            45
Lys Cys Val Asn Pro Leu Thr Thr Ala Ser Gly Pro Leu Gln Leu Lys
                    55
  50
                                        60
Val Gly Ser Ser Leu Thr Val Asp Thr Ile Asp Gly Ser Leu Glu Glu
                  70
                                     75
Asn Ile Thr Ala Ala Ala Pro Leu Thr Lys Thr Asn His Ser Ile Gly
              85
                                90
Leu Leu Ile Gly Ser Gly Leu Gln Thr Lys Asp Asp Lys Leu Cys Leu
           100
                              105
Ser Leu Gly Asp Gly Leu Val Thr Lys Asp Asp Lys Leu Cys Leu Ser
                         120
Leu Gly Asp Gly Leu Ile Thr Lys Asn Asp Val Leu Cys Ala Lys Leu
   130
                      135
                                         140
Gly His Gly Leu Val Phe Asp Ser Ser Asn Ala Ile Thr Ile Glu Asn
           150 155
Asn Thr Leu Trp Thr Gly Ala Lys Pro Ser Ala Asn Cys Val Ile Lys
                      170
              165
Glu Gly Glu Asp Ser Pro Asp Cys Lys Leu Thr Leu Val Leu Val Lys
           180
                             185
                                               190
Asn Gly Gly Leu Ile Asn Gly Tyr Ile Thr Leu Met Gly Ala Ser Glu
                         200
                                            205
Tyr Thr Asn Thr Leu Phe Lys Asn Asn Gln Val Thr Ile Asp Val Asn
                      215
                                         220
Leu Ala Phe Asp Asn Thr Gly Gln Ile Ile Thr Tyr Leu Ser Ser Leu
                 230
                                    235
Lys Ser Asn Leu Asn Phe Lys Asp Asn Gln Asn Met Ala Thr Gly Thr
              245
                               250
                                                   255
Ile Thr Ser Ala Lys Gly Phe Met Pro Ser Thr Thr Ala Tyr Pro Phe
                                                270
         260
                             265
Ile Thr Tyr Ala Thr Glu Thr Leu Asn Glu Asp Tyr Ile Tyr Gly Glu
                         280
       275
                                            285
Cys Tyr Tyr Lys Ser Thr Asn Gly Thr Leu Phe Pro Leu Lys Val Thr
   290
                      295
                                         300
Val Thr Leu Asn Arg Arg Met Leu Ala Ser Gly Met Ala Tyr Ala Met
                310
                                    315
Asn Phe Ser Trp Ser Leu Asn Ala Glu Glu Ala Pro Glu Thr Thr Glu
              325
                                 330
Val Thr Leu Ile Thr Ser Pro Phe Phe Phe Ser Tyr Ile Arg Glu Asp
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| Asp | | | 340 | | | | | 345 | | | | | 350 | | | |
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| <210: <211: <212: <213: | > 97 > DN | 2 A | .cial | . Seç | quenc | ce | | | | | | | | | | |
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| <400: atg a Met 1 | aag | cgc | | | | | | | | | | | | | | 48 |
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| att i Ile i | tcc Ser | cca Pro 35 | aat Asn | ggc Gly | ttc Phe | aca Thr | caa Gln 40 | agc Ser | cca Pro | gac Asp | gga Gly | gtt Val 45 | ctt Leu | act Thr | tta Leu | 144 |
| aaa t Lys (| tgt Cys 50 | tta Leu | acc Thr | cca Pro | cta Leu | aca Thr 55 | acc Thr | aca Thr | ggc Gly | gga Gly | tct Ser 60 | cta Leu | cag Gln | cta Leu | aaa Lys | 192 |
| gtg (Val (65 | gga Gly | Gly ggg | gga Gly | ctt Leu | aca Thr 70 | gtg Val | gat Asp | gac Asp | act Thr | gat Asp 75 | ggt Gly | acc Thr | tta Leu | caa Gln | gaa Glu 80 | 240 |
| aac Asn | ata Ile | cgt Arg | gct Ala | aca Thr 85 | gca Ala | ccc Pro | att Ile | act Thr | aaa Lys 90 | aat Asn | aat Asn | cac His | tct Ser | gta Val 95 | gaa Glu | 288 |
| cta Leu | tcc Ser | att Ile | gga Gly 100 | aat Asn | gga Gly | tta Leu | gaa Glu | act Thr 105 | caa Gln | aac Asn | aat Asn | aaa Lys | cta Leu 110 | tgt Cys | gcc Ala | 336 |
| aaa Lys | | | | | | | | | | | | | | | | 384 |
| gat Asp | | | | | | | | | | | | | | | | 432 |
| caa Gln 145 | att Ile | gtg Val | gaa Glu | aac Asn | act Thr 150 | aat Asn | aca Thr | aat Asn | gat Asp | ggc Gly 155 | aaa Lys | ctt Leu | act Thr | tta Leu | gta Val 160 | 480 |
| tta Leu | gta Val | aaa Lys | aat Asn | gga Gly 165 | Gly aaa | ctt Leu | gtt Val | aat Asn | ggc Gly 170 | tac Tyr | gtg Val | tct Ser | cta Leu | gtt Val 175 | ggt Gly | 528 |

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| gta Val | tca Ser | gac Asp | act Thr 180 | gtg Val | aac Asn | caa Gln | atg Met | ttc Phe 185 | aca Thr | caa Gln | aag Lys | aca Thr | gca Ala 190 | aac Asn | atc Ile | 576 |
|------------|--------------------------------------|-------------------|-------------------|------------|------------|-------------------|-------------------|-------------------|------------|------------|-------------------|-------------------|-------------------|------------|------------|-----|
| caa Gln | tta Leu | aga Arg 195 | tta Leu | tat Tyr | ttt Phe | gac Asp | tct Ser 200 | tct Ser | gga Gly | aat Asn | cta Leu | tta Leu 205 | act Thr | gag Glu | gaa Glu | 624 |
| tca Ser | gac Asp 210 | tta Leu | aaa Lys | att Ile | cca Pro | ctt Leu 215 | aaa Lys | aat Asn | aaa Lys | tct Ser | tct Ser 220 | aca Thr | gcg Ala | acc Thr | agt Ser | 672 |
| | act Thr | | | | | | | | | | | | | | | 720 |
| | ttc Phe | | | | | | | | | | | | | | | 768 |
| tgt Cys | tac Tyr | tac Tyr | atg Met 260 | act Thr | agt Ser | tat Tyr | gat Asp | aga Arg 265 | agt Ser | cta Leu | ttt Phe | ccc Pro | ttg Leu 270 | aac Asn | att Ile | 816 |
| | ata Ile | | | | | | | | | | | | | | | 864 |
| | caa Gln 290 | | | | | | | | | | | | | | | 912 |
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| | gac Asp | | taa * | | | | | | | | | | | | | 972 |
| <21 <21 | 0 > 44 1 > 32 2 > Pl 3 > A: | 23 RT | icia | l Sed | quen | ce | | | | | | | | | | |
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| | 0> 4 Lys | | Ala | Arg | Pro | Ser | Glu | Asp | Thr | Phe | Asn | Pro | Val | Tyr | Pro | |
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| Ile | Ser | Pro 35 | 20 Asn | Gly | Phe | Thr | Gln 40 | 25 Ser | Pro | Asp | Gly | Val 45 | 30 Leu | Thr | Leu | |
| Lys | Cys 50 | | Thr | Pro | Leu | Thr 55 | | Thr | Gly | Gly | Ser 60 | | Gln | Leu | Lys | |
| Val 65 | Gly | Gly | Gly | Leu | Thr 70 | | Asp | Asp | Thr | Asp 75 | | Thr | Leu | Gln | Glu 80 | |
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Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
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                                               125
Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys
                      135
                                         140
Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val
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Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile
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Gln Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu
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Ser Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser
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Pro Phe Asn Thr Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile
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aaaacaccca gaaaaccgca cgcgaaccta cgcccagaaa cgaaagccaa aaaacccaca 33420
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<213> Artificial Sequence
<223> Penton 1 Oligonucleotide
<400> 57
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| cgcggaagag aactccaacg cggcagccgc ggcaatgcag ccggtggagg acatgaa | 57 |
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| <220> <223> Penton 2 Oligonucleotide | |
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| <210> 59 <211> 75 <212> DNA <213> Artificial Sequence | |
| <220> <223> Penton 3 Oligonucleotide | |
| <400> 59 cgatagccgc ggctacccct acgacgtgcc cgactacgcg ggcaccagcg ccacacgggc tgaggagaag cgcgc | 60 75 |
| <210> 60 <211> 73 <212> DNA <213> Artificial Sequence | |
| <220> <223> Penton 4 Oligonucleotide | |
| <400> 60 teagegeget teteeteage eegtgtggeg etggtgeeeg egtagteggg eaegtegtag gggtageege gge | 60 73 |
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| <220> <223> Hexon Forward Primer | |
| <400> 61 cttcgatgat gccgcagtg | 19 |
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| <220> <223> Hexon Reverse Primer | |
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| <210> 63 <211> 25 | |

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<223> P-0005/U primer
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<212> DNA
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<223> P-0006/L primer
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<223> 35FMun primer
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| <400> agcaat tgtaat | 68 tgaa aaataaacac gttgaaacat aacacaaacg attctttagt tgtcgtcttc gtaa gaa | 60 73 |
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| <400> agcaat | 69 tgaa aaataaacac gttg | 24 |
| <210> <211> <212> <213> | 20 | |
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| <400> gaacag | 70 gagg tgagcttaga | 20 |
| <210><211><212><213> | 42 | |
| <220> <223> | Primer P2 | |
| <400> gttagg | 71 tgga gggtttattc cggtccacaa agttagctta tc | 42 |
| <210> <211> <212> <213> | 42 | |
| <220> <223> | Primer P3 | |
| <400> gataaq | 72 ctaa ctttgtggac cggaataaac cctccaccta ac | 42 |
| <210><211><212><213> | 20 | |
| <220> <223> | Primer P4 | |
| <400> gtggca | 73 ggtt gaatactagg | 20 |
| <210> <211> | | |

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<223> Primer P6
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<210> 76
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<223> Primer P7
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<223> Primer P8
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<211> 10
<212> PRT
<213> Artificial Sequence
<220>
<223> cRGD peptide
<400> 78
His Cys Asp Cys Arg Gly Asp Cys Phe Cys
<210> 79
<211> 32
<212> DNA
<213> Artificial Sequence
<220>
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<223> P-0011/U primer
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<223> P-0012/L primer
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gtagcaaaat aca
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<223> Native Ad37 N-terminus
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Met Ser Lys Arg Leu Arg Val Glu
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Met Lys Arg Ala Arg Pro Ser Glu
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gacaaactet tegeggtett teeagtacte ttggategga aaccegtegg ceteegaaeg 1140
agatecgtac tecgeegeeg agggacetga gegagteege ategacegga teggaaaace 1200
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<223> Primer F16 3'
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<210> 91
<211> 34
<212> DNA
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<223> Primer F35 3'
<400> 91
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<212> DNA
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<223> Plasmid p5FloxHRF
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gcacagcacc acaatattgt tcaaaatccc acagtgcaag gcgctgtatc caaagctcat 180
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tegeacgtaa eteaegttgt geattgteaa agtgttacat tegggeagea geggatgate 660
ctccagtatg gtagcgcggg tttctgtctc aaaaggaggt agacgatccc tactgtacgg 720
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